Chronic Aluminum-Induced Motor Neuron Degeneration:
Clinical, Neuropathological and Molecular Biological Aspects

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ABSTRACT: The monthly intracisternal inoculation of young adult New Zealand white rabbits with low-dose (100 µg) aluminum chloride induces aggregates of phosphorylated neurofilament that mimics the intraneuronal inclusions of amyotrophic lateral sclerosis. The chronic progressive myelopathy and topographically-specific motor neuron degeneration that occurs in the absence of suppressions of neurofilament messenger RNA levels in this model contrasts with the acute fulminant encephalomyelopathy and nonspecific gene suppressions that occur subsequent to high-dose (1000 µg) aluminum chloride inoculations. Further analysis of this unique model of chronic motor system degeneration can be expected to provide additional insights into the pathogenesis of amyotrophic lateral sclerosis.

RÉSUMÉ: Dégénérescence chronique du neurone moteur induite par l'aluminium : aspects cliniques, neuropathologiques et biologie moléculaire. Une inoculation mensuelle intracisternale d'une petite dose de chlorure d'aluminium (100 µg), chez de jeunes lapins blancs adultes de Nouvelle Zélande, induit l'apparition d'agrégats de neurofilaments phosphorylés qui simulent les inclusions intraneuronales de la sclérose latérale amyotrophique. La myélopathie progressive chronique et la dégénérescence du neurone moteur à topographie spécifique, qui survient en l’absence de suppression des niveaux d’ARN messager du neurofilament dans ce modèle, contraste avec l’encéphalomyléopathie aiguë fulminante et la suppression génique non-spécifique qui surviennent suite à l’inoculation de hautes doses de chlorure d’aluminium (1000 µg). Une analyse plus poussée de ce modèle unique de dégénérescence chronique du système moteur laisse présager qu’on en tirera une compréhension additionnelle de la pathogénèse de la sclérose latérale amyotrophique.


Amyotrophic lateral sclerosis (ALS) is a relentlessly progressive, uniformly fatal disorder of the neuronal cytoskeleton affecting the upper and lower motor neurons. The disease has a world-wide distribution and increasing age-related incidence rates of 1.6 to 2.4/100,000.1-4 Death occurs within 2.5 years in 50% of cases and 90-95% are deceased within the first decade.5,6 As a direct consequence of our studies of ALS in the Western Pacific, where original incidence rates were at least 50 times higher than worldwide rates,7-10 we have undertaken the long-term development of models of chronic, experimentally-induced neuronal degeneration, both in vivo and in vitro, in an attempt to understand the cellular and molecular pathogenic mechanisms of ALS.

Irrespective of the clinical variant studied (classical sporadic, familial or Western Pacific), the neuropathological hallmark of ALS is a loss of upper and lower motor neurons that is topographically specific (e.g. sparing of Onuf’s nucleus and cranial nuclei III, IV, VI).11 Whereas neuronal loss is a later event in the disease process, intracytoplasmic inclusions and neuroaxonal swellings consisting of interwoven skeins or parallel arrays of morphologically normal phosphorylated neurofilament within degenerating motor neurons are invariably early neuropathological findings.12-21 While there are additional neuropathological features that further distinguish between the clinical variants (e.g. paired helical filaments in Western Pacific ALS;22,23 Clarke’s nucleus and dorsal spinocerebellar tract degeneration in some cases of familial ALS),24,25 these inclusions suggest that ALS is a cytoskeletal disorder with an impairment in biosynthesis or catabolism of the neurofilament triplet protein as a common biological process underlying all of the clinical variants. Extensive epidemiological, genetic, cellular and molecular studies of the Western Pacific foci of ALS have provided insights into this process and strongly implicate environmental factors in its etiology, specifically the interaction of calcium and aluminum in the disease process.9-10 The resulting hypothesis that an environmental deficiency of calcium coupled with high aluminum...
induces a form of secondary hyperparathyroidism accompanied by enhanced gastrointestinal absorption of aluminum has been supported by a large body of data over the past three decades, as well as by the experimental induction of neuropathological lesions reminiscent of those seen in ALS in nonhuman primates (cynomolgus monkeys and Japanese macaques) chronically fed a hypocalcemic, aluminum-supplemented diet.26-28 Experimentally, although aggregates of phosphorylated neurofilament with ultrastructural characteristics reminiscent of ALS can be induced with single inoculations of large (1000 µg or greater) doses of organic or inorganic aluminum compounds in a variety of experimental hosts, these conventional models of aluminum neurotoxicity are inappropriate for the study of the process of neurofilamentous degeneration in a chronic neurodegenerative disorder such as ALS.29 Invariably, these “acute” models are accompanied by a fulminating encephalopathy marked by seizures, quadraparesis and death (10 to 14 days post inoculation) and a diffuse, nonspecific neuronal degeneration with suppression of gene transcription not thought to occur in ALS.30-33 To circumvent these difficulties, we have developed an experimental model of a slowly progressive, aluminum-induced myelopathy that mimics to a large extent the clinical and topographic specificity of motor system degeneration in human ALS. Following repeated intracisternal inoculations of low dose (100 µg) AlCl3 at 4 weekly intervals over the course of eight months in young adult New Zealand white rabbits (age 8-9 weeks), encephalopathic signs, widespread neuronal degeneration and suppressions of neurofilament mRNA levels seen in the “acute” model do not occur.34 35

Neuropathologically, there was extensive degeneration of spinal motor nuclei with argyrophilic globular inclusions within motor neuron perikarya, dendrites and axonal processes. Neurofibrillary tangle-like argyrophilic inclusions were consistently present in the giantocellularis, reticularis, raphe and trapezoid nuclei, but only rarely present in the dorsal and ventral subiculum, parasubiculum and anterior thalamus, and never found in the cerebral cortex, substantia nigra, locus ceruleus, or cerebellum. Ultrastructurally, these inclusions consisted of straight or interwoven skeins of 10 nm filaments.

Immunocytochemically, the neurofilamentous inclusions demonstrated a spectrum of immunoreactivity with monoclonal antibodies against phosphorylated and nonphosphorylated epitopes of neurofilament subunit proteins - features reminiscent of the immunohistochemical studies of ALS reported by Schmidt et al.18 While many inclusions are intensely immunoreactive with antibodies recognizing phosphorylated epitopes of neurofilament, inclusions are also present which demonstrate no immunoreactivity or are only faintly immunoreactive. Some inclusions react only to antibodies recognizing nonphosphorylated epitopes. Axonal spheroids and a variable number of intracytoplasmic inclusions (< 20%) are also uniquely immunoreactive to SMI 34, an antibody which recognized an “age-related” phosphorylation epitope of neurofilament but does not recognize acute aluminum-induced neurofilamentous inclusions.37 Molecular studies using our model of chronic aluminum intoxication demonstrated that alterations in gene transcription do not occur - unlike that reported for the acute model. In preliminary experiments, we have correlated the dose of AlCl3 administered (1000, 750, 500, 200 or 100 µg AlCl3 intracisternal-
Future in vivo and in vitro studies should continue to address the cellular and molecular mechanisms of chronic low-dose aluminum intoxication leading to the induction of neurofilamentous inclusions. Such studies should not only include attempts to understand the molecular biology of altered neurofilament gene expression, transcription, translation and post-translational modification, but also the identification of aluminum transport mechanisms, neuronal metalloenzyme receptor sites and the effects of various aluminum species, chemical remodelling of aluminum complexes and calcium-aluminum interactions of the induction of neuronal degeneration.

REFERENCES


