Metals in Motor Neuron Diseases

PER M. ROOS,*,† OLOF VESTERBERG,‡ AND MONICA NORDBERG*,[1]

*Institute of Environmental Medicine, Karolinska Institutet, SE-171 77, Stockholm, Sweden; †Department of Clinical Neurophysiology, Ullevål University Hospital, Oslo, Norway; and ‡Faculty of Natural Sciences, Uppsala University, Sweden

Degenerative processes within the nervous system are common features in disease entities such as dementia of Alzheimer type (DAT), Parkinson disease (PD), and amyotrophic lateral sclerosis (ALS). ALS is a neurodegenerative disease with unknown etiology; widespread muscle wasting and respiratory failure lead to death within a few years. Denervation can be detected with electromyography and axonal deterioration monitored by motor unit number estimates. Several suggestions about the cause of ALS have emerged but no solid theory has yet precipitated. Lead or mercury exposure has been suggested. Exposure data alone cannot support this connection. Alterations in metal kinetics may underlie the deterioration of motor function observed in patients with ALS. In this review the role of metals in motor neuron disease is discussed. Both classic studies on exposure and recent understanding of metal binding proteins are considered. Aspects of peak exposure and excretion are merged toward an understanding of metal dynamics in ALS. An overview of chemical and electrophysiological investigations is given in the context of neurodegeneration. Exp Biol Med 231:1481–1487, 2006

Key words: neurodegenerative disease; motor neuron disease; metal; electromyography; metallothionein; cerebrospinal fluid

Introduction

Amyotrophic lateral sclerosis (ALS) can be considered a model disorder for neurodegeneration. Deterioration of anterior horn cells in the spinal cord leads to loss of muscle strength and respiratory problems, with fatal outcome. Both genetic and environmental etiologies to ALS have been discussed. Animal models exist for genetic forms of ALS, as well as evidence for various exposures in connection with the disease. Oxidative damage to proteins has been proposed to cause ALS. Adverse effects of metals exhibit symptoms similar to those of ALS. Genetic variations in the metalloproteins and some enzymes responsible for handling and excretion of metals may account for the differences in disease development among individuals suffering from similar metal exposure. The role of the small metallothionein (MT) protein is discussed.

Clinical Features of ALS

ALS affects the spinal cord with an indolent and slow degradation of anterior horn cells and, subsequently, brain motor cortex cells. Progressive weakness and wasting of limb muscles is followed by respiratory problems, and the patient succumbs due to respiratory failure. Survival time from diagnosis is around 30 months without artificial ventilation, peak age of onset is 54 years, average time from onset to diagnosis is 14 months, and the incidence of ALS worldwide is 2/100,000. Verified epidemiologic data over 2 decades indicate an increasing incidence and prevalence of ALS (1).

Theories of Proposed Etiologies of ALS

A disorder with an invariably lethal course that is resistant to all possible forms of therapeutic intervention evokes many theories concerning etiology. An electrophysiological understanding of the pathological physiological mechanisms underlying the disorder is necessary in etiological discussions (2). Environmental causes have been discussed for many decades (3). Despite a massive worldwide scientific effort, few substantial clues have emerged since the original description by Charcot more than a century ago (4).

Genetic Factors. ALS has been described as a number of illnesses defined from the time of onset to fatal outcome. Familial ALS (FALS) seems to be the most rapidly developing form, and a genetic factor has been considered. Genetic alterations affecting the copper/zinc (Cu/Zn) superoxide dismutase (SOD) protein accounts for about 10% of ALS cases described as FALS. More than 90 individual mutations in SOD1 have been described as being responsible for FALS (5). No evidence exists for genetic causes of sporadic ALS, which has shown a steadily increased mortality frequency throughout the century (6).

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[1] To whom correspondence should be addressed at Institute of Environmental Medicine, Karolinska Institutet, SE-171 77, Stockholm, Sweden. E-mail: monica.nordberg@ki.se

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This increase may reflect increased awareness and improved access to diagnostic facilities such as electromyography (EMG). The increase is, however, of a magnitude that excludes genetic migration and has been interpreted from epidemiologic data alone to support an environmental etiology (1).

Viral. Viral etiologies have been thoroughly investigated. Herpes virus type 8 has been associated with ALS in some studies, although these links remain to be proven. Recent efforts to detect enterovirus, including poliovirus in ALS by reverse transcription–polymerase chain reaction, have failed. An association between some motor neuron diseases and human immunodeficiency virus (HIV) infection is not coincidental, but pathogenetically related and ALS-like disorders may perhaps be considered an HIV-related neurological complication (7).

Inflammatory. Inflammatory etiologies, including microglial activation, have been proposed. In particular, the actions of cyclo-oxygenase-2 and prostaglandins in central nervous system (CNS) inflammation have recently gained much attention. Prostaglandins act as anti-inflammatory molecules to reduce the production of nitric oxide and proinflammatory cytokines, and to increase the expression of anti-inflammatory cytokines (8, 9). Similarities between ALS and multiple sclerosis (MS) have been emphasized by some authors who discuss common mechanisms of axonal degradation (10). A high correlation between mortality due to MS and ALS exist as judged from Swedish epidemiological data (11); however, no common etiopathological theory has emerged.

Oxidative Damage. Postmortem studies have proposed oxidative injury by oxidative damage to proteins, lipids, and DNA, although the initiating causes of these events have not been identified (12).

Toxic Agents. Toxic substances of many kinds have been suggested to cause ALS, including pesticides and herbicides, rotenone, cocaine, amphetamine, and electrical injury, as well as cockpit occupation (13). However, a recent study by the ALS CARE study group could not confirm heavy metal exposure at work as a significant risk factor for ALS (13).

Several other etiological aspects have emerged, including affections of cell organelles. In summary, the evidence for persistent viral infection, glutamate-mediated excitotoxicity, oxidative stress, altered neurofilament and peripherin expression, disrupted axonal transport, neurotrophin deficiency, and mitochondrial alterations may all need consideration. As yet, no consensus has been achieved on the mechanisms that lead to selective motor neuron death, and the underlying causes are still unknown for the vast majority of patients. Further clues about genetic susceptibility and environmental triggers are urgently needed to increase knowledge about the pathogenesis, which may help in the development of prevention and more effective treatment for ALS (14).

Metal Metabolism in Dementia of Alzheimer-Type and in Parkinson Diseases

Progressive cognitive impairment is characteristic in dementia of Alzheimer-type (DAT). Pathological changes include extensive neuronal loss and the presence of neurofibrillary tangles and senile plaques in the brain. The senile plaques contain amyloid fibrils derived from a 39– to 43–amino acid peptide referred to as beta-amyloid, or Abeta. In some cases DAT is believed to be an accelerated form of aging in which a number of specific sites of the brain have in some individuals been damaged due to production of free radicals formed during normal metabolism impaired by metals. The brain would thus then be more susceptible due to low concentrations of antioxidants.

Copper and iron levels both show marked increases with age and may adversely interact with the Abeta peptide causing its aggregation as well as production of neurotoxic hydrogen peroxide (H_2O_2), contributing to the pathogenesis of DAT. Amyloid precursor protein possesses copper/zinc binding sites in its amino-terminal domain and in the Abeta domain. In patients with DAT, a 3-fold increase in oxidative damages of mitochondrial DNA compared with that of healthy subjects has been noted. Influences from exposure to certain metals such as aluminum and lead have been suggested. Metals can cause reactive oxidative stress, which can damage DNA, enzyme systems, and cellular membranes. An increased concentration of copper in cerebrospinal fluid (CSF) but a normal concentration in plasma has been found in some patients with Alzheimer disease (15). Metal–protein interactions have been pointed out in the discussion of DAT pathogenesis. Redox active metals such as copper, iron, and zinc are involved in the pathophysiology of Alzheimer disease. Transition metal imbalances in the brain of patients with Alzheimer disease, such as decreased copper and increased iron, zinc, and manganese have been reported. Iron and copper concentrations increase with normal aging in several tissues (e.g., brain), but zinc levels show either a slight decrease or they remain unchanged. Recent developments in magnet resonance spectroscopy have been useful in measuring metal contents in the human brain (16). This may contribute to a better understanding of the roles of metals.

Parkinson disease has been suggested to be related to disturbed metabolism of manganese. Parkinson-like symptoms have been described in association with an accumulation in the brain of manganese from inhalation of manganese-containing dust (17). [^{11}C]-nomifensine was reduced within the striatum of monkey following sc injections of manganese oxide. This supports the suggestion that dopaminergic nerve endings degenerate during manganese intoxication (18). Iron dysregulation and deviations in the metabolism of copper have also been discussed in connection with Parkinson disease.

Diagnosis of ALS by Electrophysiological Methods

Early signs of axonal degradation can be detected with electrophysiological methods. Both measurements of motor nerve conduction velocities and motor amplitudes and EMG are needed for proper diagnosis. In EMG, a 0.9-mm-diameter concentric needle electrode is inserted into a skeletal muscle. Muscle action potentials are amplified and measured continuously during weak and strong muscle contractions, and in the resting state. From the configuration, frequency, amplitude, and duration of the muscle action potentials detailed conclusions concerning muscle and nerve function can be drawn. EMG confirms and documents the existence of fibrillation potentials and positive sharp waves as objective measures of denervation in ALS. These potentials often can be documented many months or even years before clinical onset of the disorder. Fasciculation potentials may also be recorded and documented with EMG well before clinical fasciculation is noted by the patient. Later in the course of ALS reinnervation occurs simultaneously with denervation. Both denervation and reinnervation can be documented and quantified with EMG. For adequate diagnosis and exclusion of differential diagnoses, sampling of several muscles from different levels of the nervous system, including cranial nerve innervated muscles, is necessary.

Motor nerve conduction velocities are unaffected, as are motor amplitudes in early stages of the disease. Sometimes the early anterior horn cell degradation can be spotted by reduced persistence and prolonged latencies in the F responses from motor nerves (19). Sensory nerve conduction velocities and amplitudes are unaffected throughout the disease.

With disease progression motor amplitudes are reduced in concert with visible muscle wasting and reduced muscle strength. Clinical evaluation using the standardized Arlie-House criteria of diagnosis (20) can define disease stages. Torque measurements of muscle strength and respiratory function evaluations may also be applied. Estimates of the number of surviving motor units at a given time can be calculated. Motor unit number index (Munix) (21) is useful for monitoring disease progression and may to a limited extent aid in prognostic considerations. Munix can also be used to quantify effects of drug trials in ALS.

Among differential diagnoses, multifocal motor neuropathy with conduction block (MMN), motor axonal polyneuropathy, and late stages of myopathy are those most often considered. No biochemical markers for the disease exist and the diagnosis thus relies on combined clinical and electrophysiological data.

ALS Is a Multisystem Disorder

In addition to the obvious muscle atrophy and signs of denervation found in electromyographic investigations, pathological changes from other organ systems have been observed in ALS. The skin of patients loses elastic properties compared with that of controls. Dermal changes with atrophy of the epidermal layer, separated collagen fibrils, and accumulation of amorphous material between collagen bundles have been observed in ALS and correlated with aluminum exposure (22). Several authors report changes in microglia activation and expression of cytokines related to the immune system (9, 23). Alimentary problems are not common among patients with ALS; however, evidence exists of specific but mild liver dysfunction. At the ultrastructural level changes in hepatocyte mitochondria and the existence of paracrystalline inclusions have been found in ALS (24). Psychiatric symptoms are not frequent in ALS and depression is not found, neither at onset nor in late stages of the disease. However, combined single photon emission computed tomography and memory test studies have unveiled subtle neuropsychological deficits in patients affected by ALS without dementia. Selective impairment of memory function has been reported, but the source of memory impairment in ALS has yet to be defined (25). Cognitive impairment is sometimes present in ALS and has been described as a pathological continuum underlying a multisystem disorder (26).

At a microscopic level several cell systems in different organs seem to be affected in ALS, which lends support to the concept of ALS as a multisystem disorder. Environmental exposure to agents accumulating in tissues over time sometimes gives rise to multisystem affections. Genetic variations impairing metal ion absorption, distribution, and excretion from cells may modify these accumulations. The possibility of alterations of metalloproteins and disturbances of their functions in hepatocytes warrants further investigations into hepatic metal kinetics in ALS.

Role of Metals in Neurodegenerative Disorders

Metals are key constituents of well-characterized metalloproteins such as hemoglobin, ceruloplasmin, and ferritin. They are often bound to protein via sulphydryl groups of amino acids such as cysteine and methionine. Body stores and the concentrations of metals such as zinc and iron in cells and body fluids are well regulated and essential to protein function, notably of enzymes. Barriers to metal entry into the nervous system exist both at the level of the blood-brain barrier and the barrier between blood and cerebrospinal fluid (CSF; Ref. 27). CSF may be considered an ultrafiltrate of blood and thus contains extracellular fluid surrounding the spinal cord, which is vital to the function of anterior horn cells. Some data exist on the metal concentrations in CSF (15, 28). Metals without known physiological functions in humans but with described neurotoxic effects are mercury and lead.

Metal accumulation may cause symptoms in the nervous system. Known examples are copper accumulation in the liver and nervous system in Wilson disease (28) and increased iron concentration in the brain in hemochromatosis (16). Such changes in metal concentrations may be

secondary to disturbed metal kinetics and protein binding. Excess lithium causes epileptiform discharges. Parkinson disease has been associated with exposure to manganese. Metalloproteins such as lactotransferrin are present in pathological lesions in some neurodegenerative disorders such as DAT, ALS, and also Down syndrome (30). Lactotransferrin binds and transports iron; aluminum may partly substitute iron, and in this context it may be noted that iron is essential for myelin formation. Feeding mice excess aluminum in the diet has been shown to cause neuronal loss in the spinal cord and brain (31). Intrathecal administration to rabbits of low doses aluminum produced ultrastructural changes in the spinal cord that were indistinguishable from those found in ALS (32). Copper and iron, both redox active metals, play important roles in many enzymes. Reactive oxygen species production will result in cellular toxicity if the concentrations of these metals are not strictly regulated. Such regulation is known as homeostasis, and provides stable concentrations of metal in cellular compartments over time. Proteins involved in Abeta production have binding sites for some of the mentioned metals and may interact with copper homeostasis.

Role of Metallothionein

MT is a low-molecular-weight protein (6–7 kDa) capable of binding seven metals such as zinc, cadmium, mercury, and copper through 20 cysteinyl groups per molecule. MT thus has an unusually high metal binding capacity. MTs consist of four major forms. MT-1 and MT-2 are present in most tissues, and MT-3 is specifically present in parts of the brain, whereas MT-4 is found in stratified epithelium. MT is an excellent biomarker for excessive uptake and exposure to metal (33).

MT-1 and MT-2 have been suggested to act as a zinc donor in biological functions. It seems probable that metal ions are exchangeable, and ions with higher affinity to MT cysteinyl residues may substitute for the already bound metal ions. Such dynamics may in pathogenesis account for some of the effects of metal multiexposure. The influence of MTs in the kinetics of metals such as cadmium, zinc, and copper is well known (34). Zinc translocated from proteins into the extracellular space may be toxic to neurons (35). However, the importance and role of MTs in the nervous system for zinc homeostasis (35) and for other metals needs to be further investigated.

MT-3 has so far only been identified with zinc as a metal component. MT-3 has not been shown to be inducible, in contrast to the other MTs. MT-3 decreases in patients with DAT and its specific role could be involvement in the formation of plaques observed in subjects with DAT. Abeta interacts with copper, iron, and zinc, and increased concentrations of reactive oxygen species can result from this interaction.

MT is a small protein with proposed functions in metal detoxification and metal storage by sequestering metals. In a mouse model of experimental inflammatory encephalitis MT has anti-inflammatory properties (36). MT production is induced by metals such as cadmium and zinc, preventing the acute toxic effects of these metals. MT messenger RNA (mRNA) concentration is accordingly a useful marker for metal toxicity (37). In brain tissue, MT concentrations are low in the uninduced state; however, metal exposure and a variety of other inducers, including cortical injury, cause an increase in MT immunoreactivity in rat neocortex (38). Enhanced immunoreactivity to MT is found at the edges of scratches in astrocyte cultures.[1] MT seems to have a protective role in the CNS (39). In animal models these MT responses to neuronal injury are paralleled by an increase in MT activity in neurodegenerative states (40).

The homeostasis of metals in the nervous system is regulated by numerous metal-inducible genes. About 1500 such genes have been identified, including the genes regulating MT synthesis. The cascades that control metal-inducible transcription of genes coding for MT have recently been identified (41). Metals of the groups 1B, 2B, and 5A in the periodic table of elements are inducers of the MT gene response, with cadmium being the most powerful one. A universal gene-response to metal exposure seems to be present and important for metal homeostasis.

Routes of Metal Uptake into the Nervous System

Metals may enter the nervous system by many routes such as inhalation, digestion, skin absorption, and retrograde axonal transport.

Inhalation of labeled mercury vapor ($^{203}Hg^0$) in a dose of 8.5 μmol/kg body weight in primates leads to absorption and distribution to internal organs. The metal is absorbed at around 80% and passes the blood-brain barrier. It has been detected by autoradiography in tissue slices with uneven distribution in the nervous system with uptake in the cortical brain, the spinal ganglia, and peripheral nerves (42). Recent re-evaluations of autoradiography documentation (42) with a new focus on the spinal cord has unveiled that after inhalation of mercury vapor the metal accumulated not only in the internal organs including liver and kidneys, but also in the spinal cord. It was more abundant in the central parts of the cord. Metal atoms inhaled in very low concentrations entering the blood from the lung could, in addition, be found at the location of the anterior horn cells in the spinal cord.[2] The distribution of metal ions differs from that of atomic metals. Mercury is rapidly absorbed into many tissues and may undergo oxidation to divalent ions. These ions are bound to sulfhydryl groups of various enzymes and other proteins including blood albumin (43).

Intranasal administration of ionized manganese in microgram doses to rats and subsequent analysis of various markers for astrocytic protein damage indicated that

[1] West AK. Personal communication.

[2] Roos PM. Inhaled metal vapor can reach anterior horn cells in the spinal cord. In preparation.

astrocytes are initial targets of manganese toxicity in the CNS (44). Administration of ions of nickel or mercury or cobalt through the same olfactory pathway showed distribution of metal in the brain (45–47). It seems to be a difference in the penetration of these different metals in that mercury sticks in the olfactory bulbs, whereas manganese passes transneuronally to secondary and tertiary olfactory neurons in the brain. Exposure of the olfactory epithelium to cadmium ions results in induction of MT in the primary olfactory neurons and a transport of the metal in these neurons as a cadmium-MT complex (48). Pulmonary exposure to metal dust containing manganese may lead to Parkinson-like symptoms (17).

The enteric uptake of metal is highly variable for different metals. Exposure often includes food with elevated metal content such as fish. Dental fillings with mercury have been shown to release mercury to the gastrointestinal tract with swallowed saliva. Accumulation of methyl mercury in seafood chains ultimately arriving in the astrocytes of our CNS is also well described (49).

Methyl mercury applied as a liquid locally to the skin spreads to the CNS and causes cerebellar symptoms. Minute amounts of methyl mercury can result in devastating neurological damage and death (50).

Retrograde axonal transport has been demonstrated for horseradish peroxidase and central projections of axons are thoroughly studied by this method. The same entry route is open for metal ions as demonstrated by applying 1 μl of mercuric chloride solution to the exposed coronal pulp and detecting the metal by autometallography from the trigeminal ganglion (51). Radioactively labeled nickel is transported at a speed of 0.13 mm/hr in primary olfactory neurons (48). By retrograde axonal transport at this slow speed metal may accumulate in remote projections originating from metal deposits in various tissues.

Noxious substances might be taken up in skeletal muscle through the motor end plates and transported through retrograde axoplasmic flow to the lower motor neurons. This entry route was investigated in experiments in humans in which the transcapillary escape of [^{125}I]labeled albumin was determined in patients with ALS and compared with controls. No elevated escape was detected. Neither did the binding of lead to albumin differ between patients with ALS and controls. However, elevated concentrations of lead have been found in CSF and blood plasma compared with concentrations in controls (52).

One single metal may gain access to the nervous system from several routes simultaneously (53).

In a limited study we performed detailed interviews of patients with ALS and controls concerning significant metal exposure. A well-defined cohort (n = 14) using the standardized Arlie House criteria (20) was studied. The results indicated increased metal exposure during the lifetime in subjects suffering from ALS compared with controls (54). This study will be expanded.

Variations in Metal Metabolism and Excretion

Most toxic metals form covalent bonds with proteins. Such metals are excreted via kidneys or liver (or both) bound to protein. Measurements of free metal ions in blood in cases of neurodegenerative disorders are often negative. Such studies typically have measured metal concentrations in whole blood or serum. Blood samples often show low metal concentrations in the red blood cell fraction, and even lower in the plasma fraction where metal is bound to plasma proteins, notably albumin.

Concentrations of MTs in the liver are induced in response to metal exposure. MT is present in elevated concentrations in kidney and liver of patients with ALS compared with controls (55). MT immunoreactivity is increased in the spinal cord of patients with ALS (56). MT mRNA is also expressed in cerebral cortex and the spinal cord (57).

It seems possible that genetic variation in inducibility and functions of these and other metalloproteins contribute to variations in excretion of metal from cells within the nervous system.

We propose that ALS is caused by adverse metal effects toward motor axons within the nervous system in a situation with altered protective function from some metalloproteins, notably MTs. This proposal is based on classic studies on metal exposure in ALS as well as recent data on MT function as indicated in this review.

Further studies on metals in ALS etiology should be directed toward the role of MTs and metal metabolism and excretion from different cells within the nervous system.

Metal Multiexposure and Synergistic Effects

Along with the rising awareness that certain metal ions may be toxic to the nervous system, some ions have been proposed as the causative agent in ALS. Evidence exists linking ALS to excess exposure to aluminum (58), cadmium (59), copper (60), iron (61), lead (52), manganese (62), mercury (51), and silica (63). These elements may be found in the air, soil, drinking water, dental fillings, food, and through occupational exposures of various kinds. They have different ways of entrance into the nervous system. Considering the complex nature of environmental and occupational metal exposure in the industrial world it is less likely that one single element could be responsible for all the diverse types of tissue damage in ALS, both at the cellular level and at the ultrastructural level, described in this review. A multimetal exposure situation may more accurately describe damage to nerve cells. Synergistic effects from many elements and failure of natural protection need attention. The MT dynamics allow for exchange of metal ions in MT molecules that regulate storage and distribution of metals (64). The proteins involved in binding, distribution between tissues, and storage and excretion of metals include SOD1, MT, glutathione, lactotransferrin, and many others, quite specific for each

element. Taken together they account for considerable normal variation within the tissue and cell distribution of metal as well as metal excretion efficiency. Genetic variation may also cause disturbances in metal kinetics as shown in the copper/zinc SOD1 models and in the variable induction of MTs in response to different metal ions. If the protective function of MT is decreased, vulnerable neurons may become open to damage from adverse effects by certain metals. The normal variation in metalloprotein function needs consideration and may account for the fact that ALS is still a rare disorder despite large parts of the global population being exposed to many metals from occupational and environmental sources.

Conclusions

Neurodegenerative disorders account for an increasing morbidity worldwide. Our interest is in ALS as a model for neurodegeneration. Its etiology is unknown despite considerable scientific effort. Genetic variations in metal metabolism and kinetics as well as environmental and occupational exposure to metals need attention in unveiling the etiology of ALS. Recent findings in chemistry and biological functions of metalloproteins are of interest in this context. Neurophysiological methods combined with biochemical studies provide data for proper understanding of the pathogenesis of ALS. Determination of metal concentrations in CSF is needed for an improved understanding of axonal damage in neurodegenerative disorders. Studies on proteins, notably MTs, in CSF, may give important information in motor neuron disorders.

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