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Mechanisms of cell death and treatment prospects in motor neuron disease

運動神經元病中細胞死亡的機制和治療的展望

Scientific evidence is emerging to indicate that motor neuron injury in motor neuron disease may reflect a complex interplay between genetic factors, oxidative stress, and imbalance of the glutamatergic excitatory control of motor neurons, which may result in damage to critical target proteins and organelles. The relative importance of these factors is likely to vary in different subgroups of patients. Protein aggregation may play a role in some forms of motor neuron injury, and the eventual demise of motor neurons may occur by a programmed cell death pathway. Advances in symptomatic therapy for patients with motor neuron disease include the development of specialist clinics, with input from multidisciplinary teams, as well as hospice care in the late stages of the disease. A number of recent therapeutic trials of potential neuroprotective drugs have been conducted, including antiglutamate, antioxidant, and neurotrophic agents. To date, only the antiglutamate agent riluzole has been shown to reproducibly prolong the survival of patients with motor neuron disease. Future therapy in motor neuron disease is likely to include a 'cocktail' of neuroprotective compounds to interfere with several molecular pathway that lead to neuronal injury. In using therapeutic strategies aimed towards retarding or arresting motor neuron disease, close attention will need to be paid to quality of life issues.

科學證據漸漸顯示在運動神經元病中，運動神經元的受傷可能反映以下幾方面之間複雜的相互作用：遺傳因素，氧化壓力及穀氨酸對於運動神經元興奮不平衡的控制，以至可能對重要的目標蛋白質及細胞器造成傷害。這些因素的相對重要性在不同病人中可能會不同。蛋白質的聚集可能對部分運動神經元的受傷起作用，而且可能因為已編制的細胞死亡程式而最終引致運動神經元的死亡。最近對於運動神經元病患者的症狀治療包括專科診療所的發展，這個診療所有來自不同學科的組別，以及對於一些末期病人的臨終照顧。近來對於幾種保護神經的藥物作了治療試驗，包括抗穀氨酸，抗氧化劑及神經營養劑。但至今，只有含有riluzole的抗穀氨酸劑才可以延長運動神經元病患者的生存期。我們展望對於運動神經元病的治療可能包括一些保護神經混合物的「雞尾酒」式治療，以介入那些可以令至神經受損的幾個分子路徑。在使用一些利用延遲或阻礙運動神經元病的策略性治療時，必須要注意病人的生活質素。

Key words:
Apoptosis;
Mitochondrial dysfunction;
Motor neuron disease;
Neuroprotein;
Oxidative stress

關鍵詞：
細胞死亡；
粒線體不正常；
運動源神經細胞病；
神經蛋白質；
氧化應激

HKMJ 2001;7:267-80

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Introduction

Motor neuron disease (MND) is one of the most common adult-onset neurodegenerative diseases. The incidence of the disease is 1 to 2 per

100 000 and is fairly uniform throughout the world, with the exception of a few high incidence foci—for example, on the Kii peninsula of Japan and on the Western Pacific island of Guam. The disease affects predominantly middle-aged and elderly individuals, with a mean age of onset of 55 years, although the disease can affect younger individuals. For reasons that are not understood, men are affected more commonly than women, with a male to female ratio of approximately 1.6:1. Motor neuron disease is sporadic in 90% of patients, but in 5% to 10% of cases, the disease is familial and usually has an autosomal dominant mode of inheritance.

Motor neuron disease causes progressive injury and cell death of the lower motor neuron groups in the spinal cord and brainstem, and usually also of the upper motor neurons in the motor cortex. The affected individual typically develops a combination of upper and lower motor neuron signs, as well as progressive muscle weakness and wasting, which are usually accompanied by pathologically brisk reflexes that eventually involve the limb and bulbar muscles. Clinical variants of the disease may affect purely the lower motor neurons (progressive muscular atrophy) or the upper motor neurons (primary lateral sclerosis). Death usually results from respiratory failure due to ventilatory muscle weakness. The rate of disease progression varies among individuals, but the average survival is only approximately 3 years from the onset of symptoms. A small percentage of individuals (approximately 10%) will have the disease for more than 10 years.

The precise causes of the neurodegenerative process seen in MND remain unknown. The selectivity of the disease process for the motor system is now recognised to be relative rather than absolute. Careful clinical and pathological studies have revealed involvement in extramotor parts of the central nervous system (CNS), such as changes in other long tracts including sensory and spinocerebellar pathways, and in neuronal groups such as substantia nigra neurons and dentate granule cells in the hippocampus. Dementia is found in approximately 2% to 3% of patients with MND, and detailed neuropsychological testing has shown that subclinical changes, particularly affecting frontal function, are more common than previously recognised. Thus, MND is now regarded as a multisystem disease in which the motor neurons tend to be first and most severely affected.[1]

One of the exciting developments in MND research in the past few years has been the emergence of good cellular and animal models of motor neuron injury (Fig 1). These models allow researchers to examine cell death pathways that lead to the degeneration of motor neurons, and to test strategies for the development of

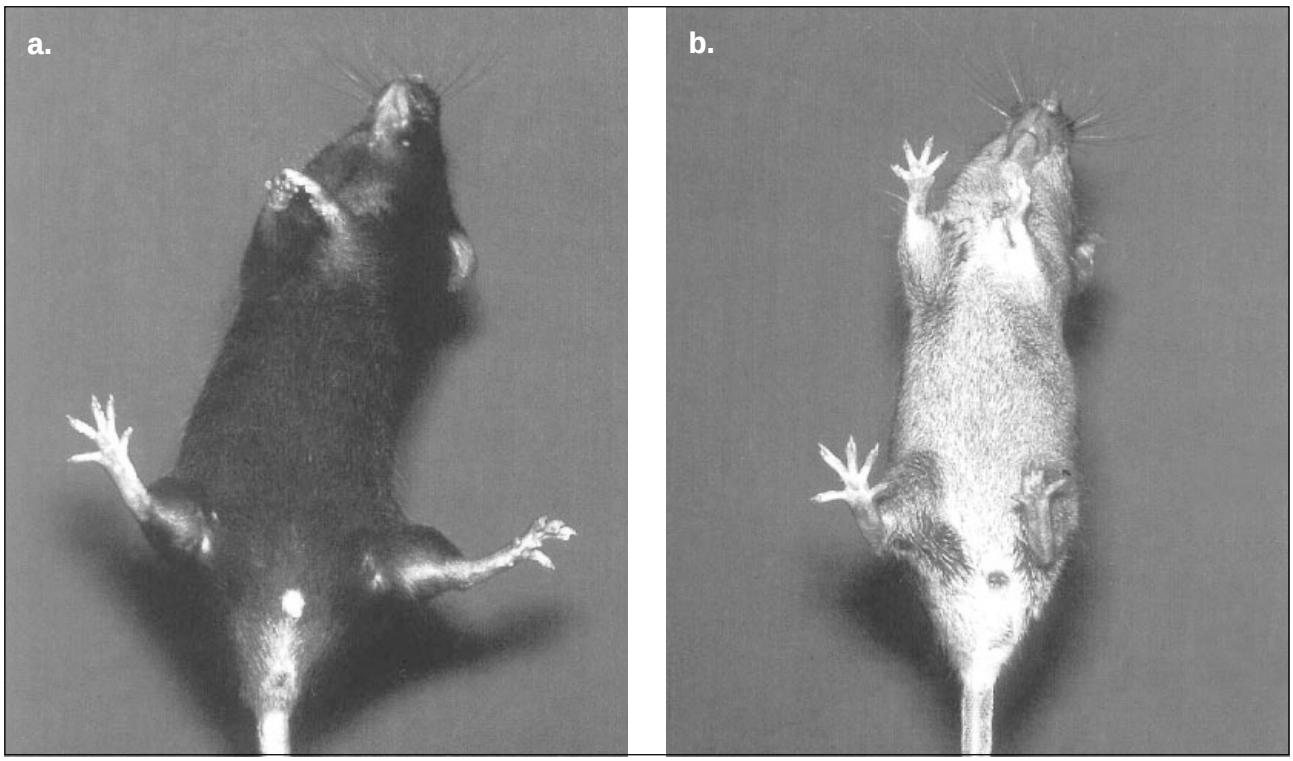

Fig 1. A normal control mouse (a) compared with a G93A copper/zinc superoxide dismutase transgenic mouse (b)
Normally, when held by the tail, the mouse shows a reflex splaying of the hind limbs. An early sign of hind limb motor dysfunction in the mutant mouse is the loss of this normal reflex

Box. Pathogenetic mechanisms that may contribute to cell death in motor neuron disease*

- Genetic factors
- Oxidative stress
- Glutamatergic toxicity
- Mitochondrial dysfunction
- Derangement of intracellular calcium homeostasis
- Damage to critical target proteins, eg neurofilaments
- Protein aggregation

* The above factors alone or in combination may lead to a programmed cell death mechanism similar to apoptosis

effective neuroprotective drugs. Neuroscientific advances are yielding insights into the mechanisms of motor neuron degeneration, as well as the cell-specific features of motor neurons that may render these cells vulnerable to disease. We are now reaching an era in which neuroprotective therapies offer the prospect of addressing the clinical and pathological progression of the disease, to an extent.

Causes of motor neuron degeneration

Current evidence suggests that multiple interacting factors contribute to motor neuron injury in MND (Box). The three key pathogenetic hypotheses include genetic factors, oxidative stress, and glutamatergic toxicity, which result in damage to critical target proteins such as neurofilaments and organelles such as mitochondria.[2-4] There is ongoing interest in the role played by protein aggregation in motor neuron injury.[5] There is also emerging evidence that motor neurons may die by apoptosis or a process of programmed cell death.[6] In relation to several of these factors, there are cell-specific features that may render motor neurons susceptible to injury.[7] These pathways leading to neuronal injury do not necessarily operate in isolation but are interlinked, with derangement of intracellular calcium homeostasis occupying a central position.

Genetic factors associated with motor neuron disease

Genetic factors associated with MND are summarised in Table 1. Motor neuron disease is sporadic in 90% of patients and familial in approximately 5% to 10%. Familial cases usually show autosomal dominant inheritance, athough autosomal recessive and X-linked inheritance may be seen in some pedigrees. It is apparent that multiple abnormal gene products can set the scene for motor neuron degeneration. A major research finding was that 20% of families with autosomal dominant MND showed mutations in the gene on chromosome 21, that encodes the free radical scavenging enzyme copper/zinc (Cu/Zn) superoxide dismutase (*SOD 1*).[8] More than 60 different mutations, mostly point mutations, have been described in approximately 250 familial MND pedigrees.[9]

Superoxide dismutase is a cytosolic metalloenzyme with copper and zinc binding sites, whose major role is to catalyse the conversion of intracellular superoxide radicals to hydrogen peroxide. The hydrogen peroxide is then in turn removed by the action of other free radical scavenging enzymes. Superoxide dismutase-1 is widely distributed in cells throughout

Table 1. Genetic factors associated with motor neuron disease

Genetic factor/disease	Gene locus
Autosomal dominant	
Mutations in copper/zinc superoxide dismutase	Chromosome 21
Juvenile onset amyotrophic lateral sclerosis	Chromosome 9q34
Amyotrophic lateral sclerosis with fronto-temporal dementia	Chromosome 9q21-q22
Gene unknown in approximately 80% of pedigrees	
Autosomal recessive	
Tunisian juvenile onset amyotrophic lateral sclerosis	Chromosome 2q33-q35
Scandinavian amyotrophic lateral sclerosis with D90A copper/zinc superoxide dismutase mutation	Chromosome 21
Juvenile onset amyotrophic lateral sclerosis	Chromosome 15q15-q21
X-linked	
X-linked dominant (single family)	Chromosome X centromere
Possible genetic risk factors for motor neuron disease (non-Mendelian inheritance)	
Deletions or insertions in amino acids lysine, serine, and proline repeat region of neurofilament heavy subunit gene	Chromosome 22q12
Excitatory amino acid transporter gene	Chromosome 11p13
Survival motor neuron gene	Chromosome 5q13
Neuronal apoptosis inhibitory protein	Chromosome 5q13
Apolipoprotein E epsilon 4	Chromosome 19q13
Cytochrome c oxidase subunit 1	Mitochondrial
Apurinic apyrimidinic endonuclease	Chromosome 14q11-q12
Manganese superoxide dismutase	Chromosome 6q25

the body. The reasons why motor neurons are selectively vulnerable to injury in the presence of *SOD 1* mutations are not yet clear, although one factor may be the high level of expression of this antioxidant defence protein by motor neurons.[10] The pathways that lead to cell death of motor neurons in the presence of *SOD 1* mutations have not yet been fully identified, although there is convincing evidence that the mutant *SOD 1* protein exerts its detrimental effects through a toxic gain-of-function rather than a loss-of-function. Several hypotheses have been formulated to explain this toxic gain-of-function.[2,4] One of the most favoured hypotheses is that the mutant *SOD 1* protein may have a more open active channel, thereby unshielding the active copper ion site. This mechanism may increase the accessibility of the active site to reactive species such as hydrogen peroxide or peroxynitrite. As a result, highly destructive hydroxyl radicals or derivatives of peroxynitrite could be formed, leading to nitration of the tyrosine residues on intracellular proteins. Two other suggestions for the toxic gain-of-function of mutant *SOD 1*, less directly linked to oxidative stress, include the formation of toxic intracellular aggregates of the mutant protein and the possibility of neurotoxicity resulting from cytosolic release of copper and/or zinc metal ions.

The genetic alterations that account for the remaining 80% of patients of autosomal dominant MND currently remain unknown. Linkage analysis has revealed that chromosomes 2q, 9q, and 15q may harbour mutations associated with rare forms of juvenile onset MND.[9,11,12] Familial MND, and associated frontotemporal dementia, have recently been linked to chromosome 9q21-q22.[13]

There have been occasional reports of genetic abnormalities found in individuals with apparently sporadic MND. Deletions or insertions in the amino acids lysine, serine, proline (KSP) repeat region of the gene encoding the neurofilament heavy protein, a major component of the neuronal cytoskeleton, have been described.[14,15] A single case of an MND phenotype with a mutation in subunit 1 of cytochrome c oxidase has been described.[16] Cytochrome c oxidase is an important component of the mitochondrial respiratory chain,[16] and of the apurinic apyrimidinic endonuclease enzyme,[17] which has a key role in the repair of oxidative damage to DNA. Some studies have indicated that the apolipoprotein 4 genotype may be a risk factor for the development of bulbar onset MND,[18] although not all research groups have been able to confirm this finding. Mutations in the glutamate re-uptake transporter protein excitatory amino acid transporter 2 (EAAT2), in the survival motor neuron and neuronal apoptosis inhibitory protein genes may occasionally be found in patients with amyotrophic lateral sclerosis or pure lower motor neuron syndromes. It has also been suggested that a polymorphism in the mitochondrial targeting sequence of manganese superoxide dismutase may be over-represented in cases of sporadic MND, a hypothesis examined in a recent review by Andersen et al.[9]

The possibility of hereditary factors protecting against the development of motor neuron degeneration has also been postulated. In Finland, the D90A *SOD 1* mutation has been shown to result in the development of MND only when the patient is homozygous for the mutation, whereas in other parts of the world D90A heterozygotes appear to develop MND. This finding has led to the hypothesis that a co-inherited neuroprotective factor may be present in Finnish patients.[19]

Cross-mating experiments using transgenic mice expressing human *SOD 1* mutations have also demonstrated that certain genetic manipulations can alter the time of onset or disease course of motor neuron degeneration. Overexpression of the anti-apoptotic protein Bcl-2 appears to delay the onset,[20] while overexpression of a dominant-negative inhibitor of the interleukin-1 β–converting enzyme slows disease progression in the presence of mutant *SOD 1*.[21]

Oxidative stress

The effects of oxidative stress within non-replicating cells such as neurons may be cumulative, and injury by free radical species is a major potential cause of the age-related deterioration in neuronal function seen in several human neurodegenerative diseases. Activation of glutamate receptors and the generation of intracellular free radicals are interlinked processes, which may generate an escalating cascade of motor neuron injury.[22] There is particular interest in the role of oxidative stress in MND, given that mutations in the *SOD 1* gene, which encodes an enzyme crucial for cellular antioxidant defence, underlie some cases of familial MND as described above. The close clinical and pathological similarity between the sporadic and *SOD 1*–related familial forms of MND suggest that there may be common pathophysiological mechanisms operating.

Studies of human postmortem CNS tissue have shown the presence of biochemical changes to proteins and DNA, thereby demonstrating the effects of free radical damage. These changes are noted to be more pronounced in MND cases compared with controls.[23,24]

Other postmortem neurochemical changes, including altered expression of components of the intracellular antioxidant defence systems, have been interpreted as an attempted compensatory response to the presence of oxidative stress during the course of MND.[25,26] Fibroblasts that have been cultured from the skin of patients with familial or sporadic MND show increased sensitivity to oxidative insults compared with those from controls.[27] Recent studies of cerebrospinal fluid (CSF) from MND patients have shown an increase in levels of 4-hydroxynonenal[28] and 3-nitrotyrosine[29] compared with controls, which may reflect abnormal free radical metabolism.

Excitotoxicity

Glutamate is the major excitatory transmitter in the human nervous system, and tremendous complexity has been uncovered, both in its mechanism of action and in the molecular structure of its receptors. This diversity means that particular types of neurons, such as motor neurons are likely to express a unique profile of glutamate receptors that are suited to cell specific physiological properties. Several glutamatergic pathways impinge on motor neurons, including the corticospinal terminals, excitatory interneurons, and collaterals from the Aα fibres innervating muscle spindles. Motor neurons are activated by the stimulation of cell surface glutamate receptors; other neurotransmitters, such as thyrotropin releasing hormone (TRH) and serotonin, probably exert a modulating effect. The α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) glutamate receptor subtype is responsible for much of the routine fast excitatory neurotransmission, and motor neurons may be particularly susceptible to toxicity via activation of cell surface AMPA receptors.[30] During normal glutamatergic neurotransmission, glutamate is released from the presynaptic terminal and passes across the postsynaptic cleft to activate postsynaptic receptors. The excitatory signal is then actively removed by glutamate re-uptake transporter proteins, which are located predominantly on perisynaptic glia.

It is known that excessive stimulation of glutamate receptors can injure neurons by mechanisms such as derangement of intracellular calcium homeostasis and excessive free radical production. The term excitotoxicity has been coined for this process. A body of circumstantial evidence has implicated glutamate-mediated toxicity as a contributory factor to motor neuron injury.[3,31] The key findings are that the expression and function of the major glial glutamate re-uptake transporter protein EAAT2, may be impaired in MND and that CNS extracellular and CSF levels of glutamate may be abnormally elevated in at least a proportion of MND patients.[32-34] In addition, both positron emission computed tomography studies and transcranial magnetic stimulation of the motor cortex in patients with MND have indicated that there is hyperexcitability of the motor system.[35,36] The presence of abnormally spliced RNA transcripts for the EAAT2 protein was recently reported to be present in the postmortem spinal cord and motor cortex of patients with MND. It was suggested consequently that a specific defect of RNA processing may be an important aetiological factor in the disease.[37] Other groups, however, have shown that these alternatively spliced RNA transcripts may be found in the CNS of normal controls and are not specific for MND.[38]

Currently it remains uncertain whether any dysfunction of the glutamatergic neurotransmitter system in MND is a primary pathophysiological process or is secondary to other primary processes leading to motor neuron failure. Whether as a primary or propagating process, it seems that glutamatergic toxicity contributes to the injury to motor neurons. This suggestion is supported by the finding that anti-glutamate therapy has some effect in prolonging survival in human patients,[39] and in a transgenic mouse model of familial MND.[40]

Mitochondrial dysfunction

Important functions of mitochondria include the generation of intracellular adenosine triphosphate (ATP), the buffering of intracellular calcium, generation of intracellular free radicals, and the initiation of apoptosis. These properties are potentially relevant in the pathogenesis of MND and other neurodegenerative disorders. Mitochondrial proteins and DNA have been shown to be particularly susceptible to free radical damage and free radicals are known to inhibit the activities of specific mitochondrial enzymes. Age-related changes in mitochondrial function have been suggested as an important factor contributing to late-onset neurodegenerative diseases. A significant factor contributing to the age-related decline in mitochondrial function is the effect of accumulating mutations in the mitochondrial genome, which is especially likely to occur in post-mitotic tissues such as the CNS. Several factors may contribute to the vulnerability of mitochondrial DNA, including the very low ratio of non-coding to coding sequences compared with nuclear DNA, the proximity to the major intracellular source of free radical production, together with minimal provision of DNA repair mechanisms, and the absence of protective histones in the vicinity of mitochondrial DNA.

There is emerging evidence that mitochondrial dysfunction may contribute to motor neuron injury in MND and this has been reviewed.[41,42] The first evidence that abnormalities of mitochondria may accompany the pathological process in MND came from ultrastructural studies that showed structural changes in the mitochondria in muscle, intramuscular nerves, and liver, as well as within spinal cord motor neurons. Increases in the activities of complex I and/or complex II/III have been reported in the frontal cortex of patients with *SOD 1*–related familial MND.[43] The observed changes are thought to represent a compensatory mechanism for oxidative damage to mitochondria, and uncoupling of oxidative phosphorylation. Several studies have indicated a reduction in complex IV activity in postmortem spinal cord specimens from cases of MND. Fujita et al[44] reported a decrease in complex IV activity in MND spinal cord, especially in the ventral horn region. Borthwick et al,[45] using a histochemical technique, reported reduced complex IV activity in individual spinal motor neurons from patients with MND. Complex IV deficiency, including the presence of complex IV negative muscle fibres, has also been reported in muscle from sporadic MND cases.[46]

One patient has been described with a MND phenotype and severe deficiency of muscle complex IV, which was due to a microdeletion in subunit 1 of cytochrome c oxidase (complex IV).[16] Evidence has also been put forward to suggest that mtDNA mutations may be associated with MND. Vielhaber et al[46] screened the mtDNA in skeletal muscle from 17 MND patients and found one patient with multiple mtDNA deletions and a further 13 patients with total mtDNA levels below those of control subjects. MtDNA deletions have also been reported within the CNS. Dhaliwal and Grewal[47] measured levels of the so-called common deletion: a 4977 base pair deletion of mtDNA previously shown to be increased in the brain of aged individuals. The levels of the common deletion were higher in the motor compared with temporal cortex and an average of 11-fold higher in MND cases compared with controls. The question of whether alterations in the mitochondrial genome might be significant in terms of cellular function has been investigated by the production of cybrid cell lines. The cell lines are achieved by fusing patients' platelets, which are devoid of a nucleus, with rho 0 cells, which have been depleted of mitochondria. Any alterations in mitochondrial function are assumed to arise from differences in the mtDNA in the platelets from the patients with the disease of interest. When cybrids were created from patients with MND, several defects were observed, including altered mitochondrial ultrastructure, decreased complex 1 activity, increased expression of free radical scavenging enzymes, and altered intracellular calcium homeostasis.[48]

Further evidence for a contributory role of mitochondrial dysfunction in motor neuron injury comes from the study of cellular and animal models. A neuronal cell line transfected to express mutant *SOD 1* has been reported to show loss of mitochondrial membrane potential. In addition, an increase in cytosolic calcium concentration, suggesting a reduction in the ability of the mitochondria in these cells to sequester calcium, has been noted.[49] Studies in transgenic mouse models of *SOD 1*–related MND have shown that one of the very early features of motor neuron injury, occurring before the animals develop clinical evidence of motor neuron dysfunction, is the appearance of abnormal mitochondria.[50] The mitochondria are initially noted to be dilated, have disorganised cristae and fractured outer membranes, and eventually form vacuolar structures. The therapeutic effects of compounds that have an effect on mitochondrial function have also been investigated in the *SOD 1* transgenic mouse model. Creatine buffers energy levels within the cell, maintains ATP levels, and stabilises the level of mitochondrial creatine kinase, which inhibits opening of the mitochondrial permeability transition pore. Administration of creatine to G93A transgenic mice has been shown to improve motor function and extend survival in a dose-dependent manner, as well as causing a reduction in biochemical indices of oxidative damage in the spinal cord.[51]

Derangement of intracellular calcium homeostasis

Calcium plays a fundamental role in many normal cellular processes, including neuronal excitability and regulation of intracellular second messenger systems. The concentration of intracellular free calcium is tightly controlled in most neurons at approximately 100 nM. Intracellular calcium homeostasis is inextricably linked to other potential mechanisms underlying degeneration of motor neurons, including glutamatergic toxicity, oxidative stress, and mitochondrial dysfunction.

The possibility that spinal motor neurons may be particularly vulnerable to calcium-mediated injury is suggested by the observations that this cell population appears to lack the expression of the calcium-buffering proteins parvalbumin and calbindin D28K,[52] while expressing abundant calpain II, a calcium-activated proteolytic enzyme. Motor neuron groups, such as the oculomotor neurons, which are less vulnerable to pathology in MND, express parvalbumin. In addition,

the expression of calcium-permeable AMPA receptors by human motor neurons may be another factor contributing to the vulnerability of this cell group to calcium-mediated toxic processes.[53]

Siklos et al[54] reported increased calcium levels, increased mitochondrial volume, and increased numbers of synaptic vesicles in the motor axon terminals of patients with MND.[54] In a subsequent study, the same group demonstrated—by using the oxalate-pyroantimonate technique and ultrastructural examination—that motor neurons of transgenic mice with a G93A *SOD 1* mutation show alterations in intracellular calcium level.[55] The cell bodies and proximal dendrites of spinal motor neurons show small vacuoles filled with calcium in mice aged 60 days. In contrast, oculomotor neurons, which tend to be spared during the disease process, show only minimal evidence of degeneration, no vacuolation, and no increased calcium or altered calcium distribution. The authors concluded that the free radical–mediated stress induced by mutant *SOD 1* appeared to induce deleterious changes in intracellular calcium distribution in motor neuron populations lacking calbindin D28K and/or parvalbumin. In contrast, motor neurons possessing these calcium-binding proteins are more resistant to stress.[55]

Cytoskeletal protein defects

Neurofilament proteins form a major component of the cytoskeleton of neurons. Important functions of neurofilament proteins include the maintenance of cell shape and axonal calibre, as well as axonal transport. Neurofilaments are the most abundant structural proteins in large cells with long axons, such as motor neurons. Neurofilaments are composed of three subunit polypeptides: neurofilament light (NF-L [molecular weight, 68 kDa]); neurofilament medium (NF-M [150 kDa]); and neurofilament heavy (NF-H [200 kDa]), assembled in a 6:2:1 ratio to form macromolecular filaments. Neurofilament light makes up the core of the neurofilament, whereas NF-M and NF-H are arranged around this core and contribute to the side-arms radiating from the filament. Neurofilament subunits are assembled in the motor neuron perikaryon and are transported down the axon by slow axonal transport. Under normal circumstances, there is progressive phosphorylation of the neurofilament proteins as they are transported down the axon.

Neurofilament proteins are of interest in MND as potential subcellular targets for injury. The abnormal assembly and accumulation of neurofilaments in the cell body and proximal axons of motor neurons, is a characteristic pathological feature in MND.[56] Some studies have reported an increase in phosphorylated neurofilaments within the cell bodies of surviving motor neurons in MND, although other groups have failed to replicate this observation. Ubiquitinated inclusions with compact or Lewy body–like morphology seen in surviving motor neurons in MND may show immunoreactivity for neurofilament epitopes. In some cases of *SOD 1*–related familial MND, large argyrophilic hyaline conglomerate inclusions, expressing both phosphorylated and non-phosphorylated neurofilament epitopes, have been observed in the cell bodies and axons of motor neurons (Fig 2).[57] The importance of neurofilaments in the normal functioning of motor neurons is demonstrated by the finding that occasional cases of sporadic MND have deletions or insertions in the KSP repeat region of the neurofilament heavy gene.[14,15] In addition, pathological changes within motor neurons develop in transgenic mice overexpressing NF-L or NF-H subunits[58,59] or in mice expressing mutations in the *NF-L* gene.[60] These in vivo models demonstrate that disruption of neurofilament assembly can selectively injure motor neurons.

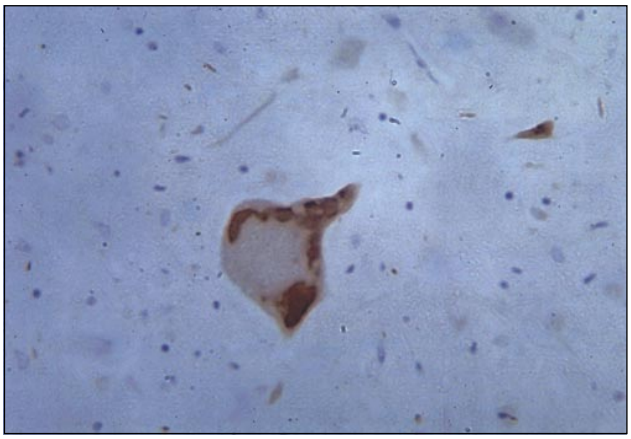

Fig 2. Argyrophilic hyaline conglomerate inclusion within a surviving motor neuron in a patient with copper/zinc superoxide dismutase–related familial motor neuron disease (I113T mutation)
The inclusion bodies show intense immunoreactivity for phosphorylated and non-phosphorylated neurofilament epitopes

Transgenic mice that carry mutations in the human *SOD 1* gene also show neuropathological changes affecting neurofilament organisation. The G93A mutant transgenic mouse has been shown to develop spheroids within motor neurons that contain both phosphorylated and non-phosphorylated neurofilaments,[61] as well as reductions in neurofilament proteins and decreased transport rates in the ventral root axons.[62]

Protein aggregation

A recurring theme highlighted in research into neurodegenerative diseases has been the misfolding of

mutant proteins and the formation of intracellular aggregates. There is continuing debate on whether such aggregated proteins play a key role in disease pathogenesis, whether they represent harmless bystanders, or indeed whether they could exert beneficial effects by acting as 'dustbins' to sequester aberrant intracellular proteins. In the *SOD 1* transgenic mouse model of familial MND, the mutant *SOD 1* protein forms prominent cytoplasmic inclusions in motor neurons and sometimes in astrocytes. These aggregates develop before the onset of clinical disease, and may represent one of the earliest identifiable signs of disease. This has led to the suggestion that protein aggregates may play a pathogenetic role in motor neuron injury.[5] There are three hypotheses to explain how mutant *SOD 1* protein aggregates could produce cellular toxicity:

(1) The aggregates of mutant *SOD 1* could sequester other proteins required for normal motor neuron function;
(2) By repeatedly misfolding, the *SOD 1* aggregates may reduce the availablity of chaperone proteins required for the folding and function of other essential intracellular proteins[63]; and
(3) The *SOD 1* mutant protein aggregates may reduce the proteosome activity needed for normal protein turnover.[64] Further research insights can be expected in this area of motor neuron protein chemistry in the near future.

Apoptosis

Apoptosis, a term used in Greek to describe the "dropping off of petals from flowers or leaves from trees" describes the controlled removal of cells by an energy-dependent cell death programme. Multiple differing molecules contribute to the control of apoptosis. Key molecular players include:

(1) The caspase family of proteolytic enzymes, which orchestrate cell destruction by destroying a number of intracellular targets such as structural and regulatory proteins;
(2) The Bcl-2 family of proteins, whose balance between pro- and anti-apoptotic members is important in regulating cell survival or destruction; and
(3) The apoptosis inhibitor family of proteins, which suppress apoptosis by preventing proteolytic activation of specific caspases.

Three main pathways triggering caspase activation have been identified including the release of pro-apoptotic factors from mitochondria, the activation of ligand receptor systems of the tumour necrosis factor family (including the Fas-Fas ligand), and the subsequent recruitment of cytosolic adaptor proteins.[6] Recently, stress to the endoplasmic reticulum has been shown to activate caspase 12. Initiator caspases cleave and activate executioner caspases, of which caspase 3 seems to be important within neurons.

There is a body of robust evidence that apoptosis occurs in developmentally regulated motor neuron death, and in the death of motor neurons following axotomy. Raoul et al[65] have recently provided evidence that programmed cell death of motor neurons during development may be triggered by caspase 8 activation, through the Fas cell surface death receptor. In a chronic neurodegenerative disease such as MND, conclusive evidence of apoptosis is likely to be difficult to detect, given the rapidity of the apoptotic cell death process in relation to the relatively slow time course of the disease. Only one in several thousand neurons might be expected to manifest ongoing signs of apoptosis in a temporally static postmortem specimen from an individual with MND.

The evidence that motor neurons may die in MND by a programmed cell death pathway has been reviewed.[6] Key points from studies of human post-mortem material include the following findings. Careful examination of spinal cord tissue from patients with MND has shown the structural morphology of degenerating motor neurons to be compatible with apoptosis, as well as internucleosomal DNA fragmentation detected by terminal deoxynucleotidyl transferase-mediated dUTP nick end-labelling staining.[66] Increased expression of certain apoptosis-related molecules including Le^y antigen and prostate apoptosis response-4 protein has been demonstrated in MND spinal cord. Several studies have shown alteration in the balance of expression and subcellular compartmental localisation of pro-and anti-apoptotic members of the Bcl-2 family in a direction favouring apoptosis in the spinal cord in patients with MND.[66,67] Significant increases in the activities of caspase 1 and caspase 3 have also been reported in the spinal cord of patients with MND compared with controls.[66,68]

Additional evidence supporting the hypothesis that motor neurons in MND may die by a programmed cell death pathway has emerged from the investigation of cellular and animal models of *SOD 1*–related familial MND. There is evidence from cellular models that mutations in *SOD 1* convert the enzyme from a protein with antioxidant, anti-apoptotic functions to a protein with apoptosis-promoting effects. Mutant *SOD 1* appears to exert its pro-apoptotic effect through a toxic gain-of-function. Neuronal cell lines expressing mutant *SOD 1* are more likely to die by apoptosis

compared with control cells, when the cellular system is subjected to stress by serum withdrawal. Using this type of experimental paradigm, caspase inhibitors can exert neuroprotective effects, as can increased expression of the anti-apoptotic protein Bcl-2.[69] Cross-breeding experiments between G93A *SOD 1* transgenic mice and transgenic strains over-expressing anti-apoptotic molecules, result in amelioration of the murine disease. There is evidence of DNA laddering, increased expression and activation of caspase 1 and caspase 3 in the spinal cords of symptomatic G93A *SOD 1* mice, along with alterations in the balance of key members of the Bcl-2 family in a direction favouring apoptosis.[68,70] Intraventricular administration of a broad spectrum caspase inhibitor delays disease onset by about 20 days, prolongs lifespan by 22%, and increases the numbers of surviving motor neurons in the cervical cord.[68]

Other pathogenetic hypotheses

Other pathogenetic hypotheses for MND include the potential contributions of exogenous toxins, viral infection, and immune-mediated mechanisms, which have been reviewed elsewhere.[71,72]

Therapeutic prospects in motor neuron disease

Advances in supportive care and symptom control

The management of MND poses considerable ethical, logistical and educational problems. Ethical issues are involved in aspects of management, including the use of artificial methods for maintaining nutrition, ventilatory support, the use of neuroprotective drugs to slow disease progression, and the use of opiate medication in the terminal phase of the disease. Logistical and educational problems arise from the relative rarity of MND and the fact that many health care professionals have little experience in dealing with the rapidly progressive bulbar weakness and respiratory failure, which may occur during the course of the disease. Coordinated action by multiple health care professionals within a multidisciplinary team, can lessen the difficulties experienced by patients with MND and their families.

Symptomatic therapy aimed at alleviating the distressing symptoms that often arise during the course of MND, can do much to improve the quality of life for patients. Detailed discussion of these therapies is beyond the scope of this article. Some of the common symptoms that may develop in patients with MND and suggested therapies are highlighted in Table 2. Many of the symptomatic therapies currently recommended by clinicians have not been assessed in rigorous controlled trials. The evidence base for many of these therapies has recently been reviewed by an American Academy of Neurology taskforce.[73]

A few areas of progress in the symptomatic management of MND are highlighted in the folowing discussion.

Specialist clinics and hospice care

The development of specialist MND clinics and multidisciplinary teams, and their inclusion of specialist nursing staff, have in my view undoubtedly improved the quality of care for patients and are of help to general practitioners and community staff who may encounter MND only rarely. The practice of utilising hospices for the care of patients with MND in the United Kingdom, has greatly improved the quality of care and support for patients in the later stages of the disease.

Nutritional management

Management of the nutritional status of MND patients has improved during the past few years. When patients first begin to develop swallowing problems, a few simple principles can help them to cope.

Table 2. Symptomatic therapy for motor neuron disease

Symptom	Therapies
Cramp	Quinine sulphate (baclofen, diazepam, phenytoin)
Fatigue	Occasionally pyridostigmine
Spasticity	Baclofen, diazepam, tizanidine
Dysphagia	Nutritional supplements, liquid thickeners, percutaneous endoscopic gastrostomy
Drooling	Hyoscine transdermal patches, atropine, amitriptyline, suction machine, parotid gland irradiation
Constipation	Ispaghula, methyl cellulose, lactulose, glycerol suppositories
Emotional lability	Amitriptyline, imipramine (l-dopa preparations)
Depression	Tricyclic agents or serotonin re-uptake inhibitors
Insomnia	Address underlying cause, fluorazepam, diphenhydramine
Pain	Address underlying cause, analgesia
Dyspnoea	Antibiotics for aspiration pneumonia, chest physiotherapy, sublingual lorazepam for acute attacks of dyspnoea. Consider non-invasive positive-pressure ventilation or other ventilatory support for selected patients. Morphine for end-stage respiratory distress

Table 3. Recent therapeutic trials in motor neuron disease and drug therapies showing a positive effect in cellular and animal models

Human Trials	
Antiglutamate agents	*Neurotrophic factors*
Riluzole	Ciliary neurotrophic factor
Gabapentin	Insulin-like growth factor 1
Lamotrigine	Brain derived neurotrophic factor
Glial-derived neurotrophic factor	
Intrathecal brain–derived neurotrophic factor—currently under analysis	
SR57746A (xaliproden [neurotrophic-like effects])—currently under analysis	
Antioxidant therapy	
N-acetyl cysteine	
Neuroprotective therapies in copper/zinc superoxide dismutase transgenic mouse model	
Vitamin E	Lyophilised red wine
Riluzole	Glial-derived neurotrophic factor
Gabapentin	Adenovirus delivered Bcl-2
Penicillamine	N-acetyl-L-cysteine
Creatine	Lysine acetylsalicylate
Janus kinase 3 inhibitor	Trientine plus ascorbate
Polyamine or putrescine modified catalase	Genistein
Ginseng root	Caspase inhibitor

Dysphagia may be increased by anxiety and social embarrassment resulting from slowness in eating, dribbling, and choking. Patients should be encouraged to eat in as relaxed and comfortable an environment as possible. Attention to food consistency is important, and the family should receive advice from a dietician. If patients continue to lose weight, then nutritional supplements of liquid or semi-solid consistency may be helpful. More active therapeutic intervention for dysphagia should be considered when the following problems are apparent:

(1) Continuing weight loss (more than 20% of the normal body weight) despite the above measures;
(2) Dehydration;
(3) Aspiration with resultant respiratory infection; and
(4) Meal times have become intolerable due to frequent choking spells.

For long-term enteral feeding, percutaneous endoscopic gastrostomy (PEG) is the procedure of choice. This is one of the major advances in symptomatic care for patients with MND. After placement of the PEG tube, many patients report great relief and increased well-being, although as yet no large scale quality of life studies have been conducted. Whether early placement of the PEG tube results in increased duration of survival has not yet been convincingly demonstrated.[74] The placement of a PEG is a relatively straight-forward procedure that can be performed under local anaesthetic. A full-strength feeding regimen that provides 1500 to 2000 calories per day can be introduced during the 48 hours following PEG tube insertion. Feeding can be managed by syringe boluses at normal meal times, or by continuous infusion by pump. The PEG procedure should not be postponed until patients have severely compromised respiratory function. At this stage in the disease, patients may develop respiratory failure following the procedure, resulting from basal atelectasis due to pressure of the air-inflated stomach against the weakened diaphragm.

Non-invasive ventilatory support measures

Management of the respiratory complications of MND include the following general measures. Attention should be given to the detection and prevention of aspiration pneumonia. Antibiotic therapy should be used at the first indication of a chest infection. Chest physiotherapy and postural drainage should be used when the patient has difficulty in clearing secretions from the chest. Patients will breathe more comfortably during sleep if placed in an upright position. When patients experience bouts of severe dyspnoea, accompanied by extreme anxiety or panic, a small dose of lorazepam (0.5-1 mg) sublingually may be helpful.[75] If breathlessness causes distress during the later stages of the disease, giving small amounts of morphine can be useful. Respiratory depression can usually be avoided if the initial dose is small and increments are gradual.

Assisted ventilation, coupled with appropriate nutritional support, could theoretically extend the patient's life indefinitely, and the implications of initiating such respiratory support must be carefully considered in discussion with each patient. There are considerable international differences in the use of assisted ventilation to manage respiratory failure in MND. The progressive nature of MND has acted as a deterrent in some countries for the active management of respiratory dysfunction for many MND

patients, particularly when other motor disabilities are extensive. Full 24-hour intermittent positive-pressure ventilation, via a tracheostomy, is an option that is chosen only rarely by fully informed patients. The costs, in terms both of financial resources and caregiver support, are substantial. Non-invasive positive-pressure ventilation (NIPPV) via a mask is a practical option for respiratory support that may be considered. Use of NIPPV support during sleep may lead to subjective improvement in sleep, resolution of morning headache, and improvement in exercise tolerance, mobility, respiratory function, and fatigue during the day.[76] More controlled trials of this form of therapy with attention to quality of life parameters are warranted.

Neuroprotective therapy

There is no therapy currently available that has a dramatic effect in slowing disease progression in MND. Some small steps have been made, however, towards this ultimate goal in the past few years. A detailed critique of all the relevant therapeutic trials is beyond the scope of this review, and only the main results will be highlighted (Table 3).[77]

Antiglutamate therapy

In 1988, Plaitakis et al[78] reported a slowing of clinical deterioration in MND patients in a pilot study of branched-chain amino acid therapy, which was postulated to modulate glutamatergic neurotransmission. This result was not confirmed, however, in a large pan-European multicentre, double-blind, placebo-controlled, randomised, parallel-group trial involving 760 patients. (Unpublished data, T Steiner).

Subsequently, other trials of antiglutamate therapy have taken place using riluzole, a sodium channel blocker that inhibits glutamate release and which has several other potentially neuroprotective effects. Two double-blind, placebo-controlled clinical trials of riluzole have been carried out among more than 1100 patients with MND.[79,39] The results of both studies showed a statistically significant, although modest, benefit in prolonging survival, and the drug has subsequently been licensed for use in patients with MND. Unfortunately, the trials did not include a quality-of-life measure, although there is no evidence that riluzole prolongs the disease stage of severe disability. In addition, in the second larger trial, no statistically significant benefit was seen in secondary parameters, including the alteration of muscle strength. Riluzole therapy is relatively expensive and the average survival benefit to be expected is modest. Against this are the counter-arguments to be weighed—that the patient population requiring the drug is relatively small, and that these patients have a lethal disease for which no other therapy is available. At present, it is unknown whether the modest overall therapeutic effect of riluzole conceals good responders and non-responders.

Gabapentin, an anticonvulsant drug with antiglutamate activity, has also been evaluated in MND. In a pilot therapeutic trial, a trend indicating potential slowing in the rate of decline of limb strength was reported. Unfortunately, this positive trend was not replicated in a further trial using higher doses of gabapentin.[80]

Antioxidant therapy

No large-scale trials of antioxidant therapy have yet been conducted among patients with MND. An underpowered trial of N-acetylcysteine therapy in 110 patients, showed a trend towards improved survival in patients with limb-onset disease, which just failed to reach statistical significance (P=0.06).[81] In the G93A *SOD 1* transgenic mouse model of MND, the antioxidant vitamin E, was shown to delay the onset of the disease, although it did not influence disease duration.[40] The effect in human patients is unproven, although many patients are prescribed vitamin E empirically, because of the emerging evidence that free radicals may contribute to motor neuron injury.

Neurotrophic factors

Multiple neurotrophic factors contribute to the health and survival of motor neurons and exert neuroprotective effects in experimental paradigms and animal models of motor neuron injury. Several neurotrophic factors, administered by subcutaneous injection, have been assessed in recent trials, so far with somewhat disappointing results. Subcutaneous ciliary neurotrophic factor showed no therapeutic benefit for patients with MND, and it actually seemed to have a detrimental effect in the high dose group.[82] Recombinant human insulin-like growth factor 1 (IGF-1) was shown to slow progression of functional impairment (assessed by the Appel ALS rating scale total score) by 26% over 9 months (P=0.01) and also slow the decline in health-related quality of life (assessed by the Sickness Impact Profile), in a trial in the United States involving 266 patients.[83] A similar trial conducted in Europe failed to replicate this result, however, and IGF-1 has not been licensed for use for MND. A trial of subcutaneously administered brain-derived neurotrophic factor showed no significant benefit, while a further trial of glial-derived neurotrophic factor administered by the intraventricular route has been aborted. Concern has been expressed about the half-life and access to motor neurons when

neurotrophic factors are administered by the subcutaneous route. Brain-derived neurotrophic factor is currently being evaluated when administered by continuous intrathecal delivery. A multinational trial of the orally active agent xaliproden (SR57746A), which seems to have neurotrophic-like effects on motor neurons, is also currently under analysis.

Experimental models

Table 3 lists pharmacological agents that have been shown to have neuroprotective effects in the *SOD 1* transgenic mouse model of motor neuron injury. The antioxidant compound, vitamin E, and two antiglutamate agents, riluzole and gabapentin, are of benefit in mutant *SOD 1* transgenic mice. In this murine model, vitamin E delays disease onset, whereas antiglutamate therapy has no effect on the timing of disease onset, although it extends survival once the disease becomes manifest. These findings suggest that oxidative stress may contribute to motor neuron injury in the early stages of MND, with glutamate toxicity playing a part later in the cell death cascade. The effects of alterations in gene expression on the clinical course of murine motor neuron disease have been investigated in several experiments, by cross-breeding *SOD 1* transgenic mice with other mouse transgenic strains. For example, the onset of motor neuron degeneration in mutant *SOD 1* mice has been retarded by concomitant overexpression of Bcl-2, a protein which inhibits apoptosis or programmed cell death.[20]

In cellular models of *SOD 1*–related MND, agents showing a positive neuroprotective action include vitamin E, increased expression of Bcl-2, inhibition of caspases, SOD mimetic compounds, the antioxidant glutathione, and copper chelators.

Future developments: a personal view

In the next few years, we are likely to see further progress in defining the molecular mechanisms of cell death underlying the neurodegenerative process in MND. It can be anticipated that further genetic mutations associated with familial MND, and the contribution of genetic factors to the sporadic form of the disease will be identified. The use of cellular and animal models of motor neuron disease will allow researchers to understand the sequential molecular events leading to motor neuron cell death and to evaluate new neuroprotective strategies. Future neuroprotective therapy for patients with MND may well involve a 'cocktail' of pharmacological agents aimed at different mechanisms contributing to the biochemical cascade of cell injury.

Measures to improve supportive care for patients and their families will continue to develop, including the judicious use of ventilatory support. As clinical and scientific developments allow the prospect of increasing the duration of the disease and prolonging survival of patients with MND, careful attention will need to be paid to the quality of life of affected individuals.

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