Amyotrophic Lateral Sclerosis

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Amyotrophic lateral sclerosis is a heterogeneous neurodegenerative syndrome that has been described in the medical literature for 150 years. Numerous theories of cause and pathogenesis have been advanced but specific mechanisms have not yet been established. What is highly likely is that numerous injurious agents can precipitate the cascade of events that results in this well-known syndrome. In the absence of knowing specific cause(s), understanding this pathogenetic mechanism may be a target for novel drug discovery efforts.

Introductory article

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Introduction

Amyotrophic lateral sclerosis (ALS), also known as motor neuron disease and *maladie de Charcot*, is a chronic, fatal, neurodegenerative disease with a prevalence of approximately 3 per 100 000 population. The cause, and consequently the cure, is not known. A number of pathogenetic theories have been proposed over the more than 150 years since ALS has been known to Western medicine. The promise of biotechnology in the twenty-first century is that secrets as to its cause(s) and rationale therapy are imminent.

The clinical hallmarks of ALS are progressive muscular atrophy and weakness, usually asymmetrical, spasticity and bulbar symptoms. Once clinically suspected, criteria establish possible, probable and definite ALS. Research is and has been directed along three fronts, as depicted in the Venn diagram in **Figure 1**.

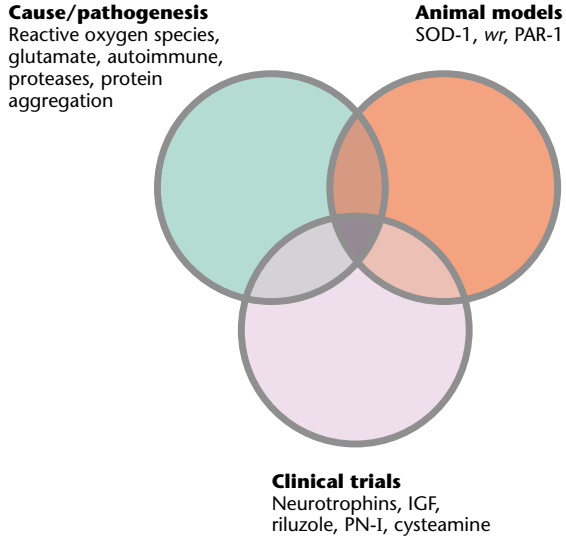

Figure 1 Venn diagram showing the three fronts along which research into amyotrophic lateral sclerosis is progressing. IGF, insulin-like growth factor; PN-I, protease nexin I; PAR-1, protease-activated receptor type 1; SOD-1, superoxide dismutase 1. The intersection represents the area for drug testing.

Motor neuron degeneration

The loss of large α motor neurons in the spinal cord ventral horn, their homologues in brainstem nuclei and of large pyramidal (Betz) cells in the motor cortex represents the principal pathological hallmark of ALS. No obvious storage product has been detected but a number of intracellular inclusions in motor neurons have been detected (**Table 1**; **Figure 2**), especially in cases of short duration and rapid autopsy. These include spheroids, conglomerates, skeins and others; they appear to be related to Golgi fragmentation found at the ultrastructural level. The second pathological hallmark is gliosis and demyelination (sclerosis) of lateral and anterior corticospinal tracts. The combination of these two pathological parameters, lower and upper motor neuron dysfunction, provides the descriptive term amyotrophic lateral sclerosis, and establishes the definitive diagnosis.

Age of onset and sex burden

Onset is variable, but the fourth or fifth decade is common for sporadic ALS, representing 90% of all reported cases. In most reports the male : female prevalence is 1.8–2.0 : 1. In genetic cases (familial ALS), age of onset appears to follow true in each generation affected. This characteristic is in contrast to some dominantly inherited neurodegenerative disorders such as the polyglutamine (CAG/Qn) expansion diseases, where CAG represents the bases coding for glutamine (Q, single letter code).

Table 1 Inclusions found in the neurons of patients with amyotrophic lateral sclerosis

Type of inclusion	Location	Staining	Significance
Spheroid	α motor neuron	Silver	Neurofilament
Conglomerate	Pyramidal Betz cell	Silver	Neurofilament plus
Bunina body	α motor neuron	Haematoxylin and eosin	Unclear
Eosinophilic	α motor neuron	Haematoxylin and eosin	Neurofilament plus
Skein	Subcortical neuron	Antiubiquitin antibody	Ubiquitin positive

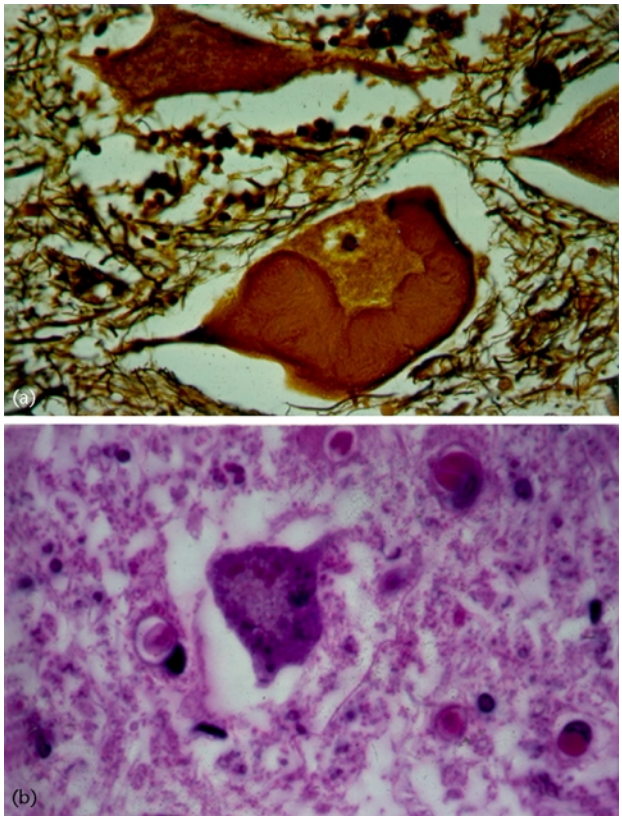

Figure 2 Inclusions found in α motor neurons in ALS spinal cord.

Progression and pathogenesis

Progression must be based on the modulation of one or more pathogenetic factors and is also variable, with both rapid and slow progressors. In sporadic ALS 50% of patients die from the disease within 5 years of diagnosis. However, despite its dire outcome and poor prognosis, ALS is a chronic disorder. Exact start dates are difficult to assess but may be 1–3 years before diagnosis.

The pathogenesis, in contrast to the specific aetiology, is likely to be similar for most cases of sporadic ALS, and possibly for familial cases as well. As described for many years, ALS is probably not a single disease but, in fact, a syndrome with multiple causes operating through a common set of mechanisms.

Familial *ALS* genetics and molecular biology

Two different genes have now been described (*ALS1*, *ALS2*) that are associated with the dominant (more common) familial ALS and with uncommon juvenile, recessive ALS (ALSJ), respectively. In 1993, the first gene, *ALS1*, was mapped to chromosome 21 and identified as C/Zn (copper/zinc) superoxide dismutase (SOD-1). The clinical pattern in such dominant families is difficult, if not impossible, to separate from that of the bulk of patients with sporadic ALS.

Superoxide dismutase

Identification of *SOD1* mutations in familial ALS rapidly followed its linkage on chromosome 21 in affected dominant families. Mutations have now been discovered in all exons of the gene; deletional mutants have not been described. Predictions as to mutation-based conformational changes disturbing the role of SOD-1 as a free radical scavenger followed. However, no significant loss of enzyme activity has been reported. Instead, genetic disease in SOD-1 familial ALS has been proposed as representing a 'toxic gain of function' model. *In vitro* studies have suggested potential alteration in free radical scavenging resulting in toxic peroxynitrite accumulation; this mechanism has not yet been confirmed.

Excitotoxicity

Another theory that has been popular in recent years is excitotoxicity based on possible increased glutamate levels in the plasma and cerebrospinal fluid of patients with ALS. A more recent extension of this theory, again unconfirmed, is a reduction in astrocytic glutamate transporters,

principally excitotoxic amino acid transporter 2 (EAAT-2) in patients with ALS (**Figure 3**).

Immunology

Although inflammation is generally considered uncommon in the spinal cord of patients with ALS, efforts to define an inflammatory component have revealed occasional cellular proliferation. In addition, humoral as well as cellular inflammatory evidence has surfaced; one group has identified antibodies that alter voltage-gated calcium channels. This effect was shown in several model systems, including model membrane systems. However, in a recent study the same crude globulin fractions that altered calcium influx could be blocked by serine protease inhibitors suggesting protease contaminated antibody factions.

Proteases and protein aggregation

This last report provided evidence for increased and/or dysregulated serine protease activity both systemically and in the spinal cord of patients with ALS. Protease dysregulation has been proposed, along with a centripetal rather than centrifugal progression, over the past 20 years. One specific protease, thrombin, has recently been explored because of its multifunctional effects (**Figure 4**) in killing motor neurons *in vitro* by activating a unique protease-activated receptor (PAR-1) that is coupled through signalling pathways that hydrolyse inositol phosphate, mobilize calcium and activate mitogen-activated protein (MAP) kinase as well as the small ras G protein, RhoA, and caspases such as caspase 3. In a classical mouse model of motor neuron generation, *wobbler* (*wr*), a dramatic (5–6-fold) overexpression of PAR-1 was found in surviving motor neurons. The *Wr* gene maps to mouse chromosome 11, syntenic with human chromosome 2p13.3, where *LMGMD2B*, the gene for a human recessive neuromuscular disease, limb girdle muscular dystrophy type 2B, maps, along with Rab-1, a small GTPase, is involved with membrane trafficking and may be associated with PAR-1 and, hence, thrombin. In a related genetic discovery, the gene for protease nexin I (PN-I), called *PI7*, maps to human chromosome 2q33-35, the same locus as *ALSJ*/*ALS2*.

The most prominent neuropathological features within surviving motor neurons in sporadic ALS, familial ALS, *wr* and *SOD1* transgenic mice are dramatic protein aggregation, precipitation or inclusion bodies. when *SOD1* mutant mice were crossed with mice transgenically overexpressing human heavy neurofilament (NF-H) subunit or with mice in which there was an absence of neurofilaments, protein aggregation was markedly reduced (65%) and survival prolonged. Crosses with NF null ($-/-$) also reduced the selective vulnerability of motor neurons. neuronal inclusions in ALS, as well as in CAG/Qn expansion diseases, Alzheimer and Parkinson diseases, appear to be the result of overexpression, alternative splicing and increased activity of tissue transglutaminase, a key proapoptotic molecule. again related to Golgi fragmentation, one group has shown that *SOD1* transgenic mice exhibit aggregates but transgenic NF-H do not, despite both showing neurofilament accumulation.

Prospects for treatment and prevention

Unfortunately, the case for the use of neurotrophic or growth factors in neurodegenerative diseases has suffered its worst track record in ALS treatment trials. Human growth hormone, ciliary neurotrophic factor and brain-

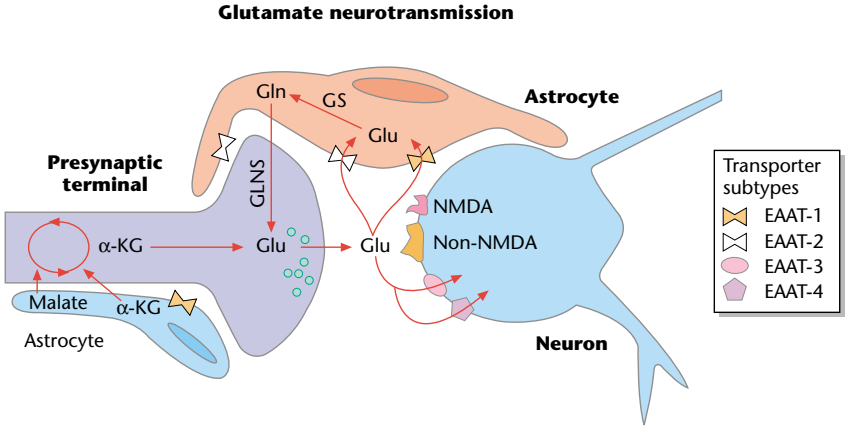

Figure 3 The glutamate hypothesis. EAAT, excitotoxic amino acid transporter; Gln, glutamine; GLNS; Glu, glutamate; GS; α-KG, α-ketoglutarate; NMDA, *N*-methyl-D-aspartate.

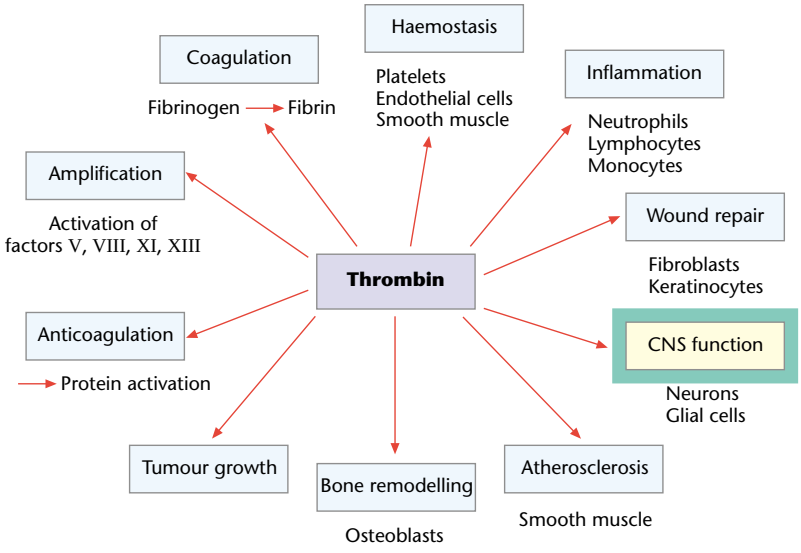

Figure 4 Multifunctional effects of thrombin. CNS, central nervous system.

derived neurotrophic factor (BDNF) have all been evaluated in large-scale controlled clinical trials and failed to show positive effects outweighing negative ones. Intrathecal BDNF may show promise in a small cohort of patients, but this is an invasive approach. Insulin-like growth factor 1 showed significant positive effects in primary and secondary efficacy measures in a North American multicentre trial, but not in a differently designed European study, and failed to gain approval from the US Food and Drug Administration. In contrast, the antiglutamate drug riluzole (Rilutek) was approved on showing a 3–4-month increase in survival and represents the only drug currently approved for use in patients with ALS. Its precise mechanism of action is unknown.

With evidence of increased levels of protease – serine (thrombin, plasmin), metallo- and cysteine aspartyl (caspases) – in neurodegenerative diseases including ALS, it is expected that pharmaceutical companies will be convinced to re-enter the ALS clinical trials arena. In addition, if protein aggregation is shown to be pathogenetic in activating the so-called caspase apoptosome, and is the result of inappropriate activation of transglutaminase, then agents that inhibit crosslinking due to this transamidating enzyme will be evaluated and further developed for the treatment of ALS and other neurodegenerative diseases.

In addition, enthusiasm about the use of embryonic stem cells has developed for a host of neurological disorders, including ALS, and laboratories are striving to understand whether such strategies will truly have a place in the therapeutic arsenal.

Summary

After more than 150 years, knowledge about ALS, or *maladie de Charcot* has certainly increased. However, key gaps in pure knowledge exist that are likely to yield to remarkable advances in biomedical technology. As ALS is a syndrome and not a disease, with probable numerous inciting agents or stimuli including genetic mutation, the mechanism(s) through which the hallmarks of the condition – motor neuron protein aggregation, degeneration and death, muscle fibre atrophy and weakness – progress should provide suitable targets for drug discovery in the near future.

Further Reading

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