JYI Journal of Young Investigators RESEARCH REVIEW

Treatment Options in Motor Neuron Disease: Amyotrophic Lateral Sclerosis and Spinal Muscular Atrophy

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Amyotrophic Lateral Sclerosis (ALS) and Spinal Muscular Atrophy (SMA) are two poorly understood Motor neuron diseases. Both diseases eventually prove fatal and their complex pathogenesis makes them difficult to treat. We reviewed the current literature to produce a comprehensive but succinct guide to ALS and SMA for the undergraduate. We review the genetics, aetiology and pathogenesis to identify similarities and differences between the two diseases. This knowledge was then used to identify and analyses current and possible future treatments. For ALS, Riluzole is currently the best, and only, licensed treatment as it increases life-expectancy by 2 to 3 months. It however only treats the symptoms. Treatments based on Vascular Endothelial Growth Factor (VEGF)'s effects on glutamate controlled calcium channels may prove beneficial in the future, as may recombinant human Insulin like Growth Factor-1 (rhIGF-1) if administration methods are improved. Because of the "gain of function" nature of ALS, prophylactic strategies involving anti-oxidant vitamins are unlikely to prove effective. Additionally, we propose a widespread involvement of Valosin Containing Protein in ALS pathogenesis. Treatment of SMA focuses mainly on raising cytosolic Full Length Survival Motor Neuron protein (FL-SMN) levels, primarily through Histone Deacetylase inhibitors, such as valproic acid, hydroxyurea and phenylbutyrate. These have shown modest benefit but significant side effects. Thyrotropine Releasing Hormone (TRH) and beta-2 adrenoceptor agonists have also been trialled, with few side effects. SMA is also a key target for gene therapy. Finally, Stem cell technology promises a cure for both conditions but, in reality, is many challenging years away from clinical application.

INTRODUCTION

Amyotrophic Lateral Sclerosis (ALS) and Spinal Muscular Atrophy (SMA) are both diseases resulting from degeneration and death of motor neurons. Each disease displays a different pattern of neural deterioration: ALS causes selective degeneration of upper and lower motor neurons, whilst SMA attacks only lower motor neurons. Both ALS and SMA result in paralysis and eventual death, commonly due to respiratory muscle failure or pneumonia.

Cases of ALS can be split into two aetiologies; 95% of cases are sporadic and 5% are familial (Rosen et al. 1993). The aetiology of familial cases is best understood and mutations in a number of genes have been found to have a direct link. The best understood locus being the SOD1 gene situated on chromosome 21 (Wulfsburg et al. 1983). This encodes superoxide dismutase, a ubiquitously expressed protein that functions as a free-radical scavenger and thus reduces oxidative cell stress throughout the body (Beckman et al. 1990; Martin et al. 2009).

Although the etiology of sporadic ALS is less well elucidated, there are a large number of hypotheses currently under examination. Glutamate neurotoxicity is one possible mechanism, as ALS sufferers have been shown to possess raised levels of glutamate in their CSF (Spreux-Varoquaux et al. 2002). As a primary site of reactive oxygen species synthesis, mitochondria are susceptible, and their dysfunction is likely to play a key part in the pathogenesis because of their involvement in glutamate excitotoxicity and oxidative stress (Depuis et al. 2004). Other possible mechanisms include multiple micro-haemorrhages due to vascular changes in the blood-brain barrier (Zhong et al. 2008) and intracellular aggregate formation (similar to Lewy body formation in Parkinson's disease) which can lead to malfunction of the endoplasmic reticulum (Shibata et al. 1994).

The pathogenesis of SMA is better understood. It is almost always caused by mutations in the survival motor neuron (SMN) gene and is inherited in an autosomal recessive manner (Lefebvre et al. 1995). The SMN gene is located on chromosome 5 and two types are present: SMN1 and SMN2. Patients with SMA lack the SMN1 gene, either due to a deletion or a mutation (Lefebvre et al. 1995). Therefore, the presence or absence of the SMN1 gene determines the phenotype. However, all patients still possess at least one copy of the SMN2 gene. The proteins expressed by this gene are similar to those produced by SMN1 and may partially or wholly rescue the cell and compensate for the loss of SMN1. The degree of compensation varies depending on the number of SMN2 genes present and therefore the SMN2 gene copy number determines the severity of the disease phenotype (Monani. 2005).

Although the genetic basis of SMA is well described, the mechanism by which the loss of SMN protein causes the disease is less well understood. There are a number of theories that include the involvement of the neuromuscular junction and

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splicing abnormalities affecting RNA processing (Felina et al. 2007; Murray et al. 2008; Monani. 2005).

The current treatment options for both diseases remain rather limited as no curative therapies are available. The present approach is predominantly to provide support and rehabilitation. This involves a multi-disciplinary team whose main focus is to maintain physical function and to supply mechanical aids where necessary to reduce any handicap. Non-invasive ventilatory support may reduce the strain on weak respiratory muscles. Overnight ventilation is commonly required due to the loss of airway tone during sleep and many patients will eventually require the use of a permanent ventilator. In the final stages, opioids and sedatives may be used in palliative care (Piepers et al. 2006).

Riluzole, an anti-excitotoxic agent is the only drug currently approved by regulating authorities for the treatment of ALS. It is thought Riluzole slows the progression of the disease by preventing glutamate excitotoxicity but it is expensive to produce and there is some controversy regarding its cost-effectiveness (Orrell. 2010).

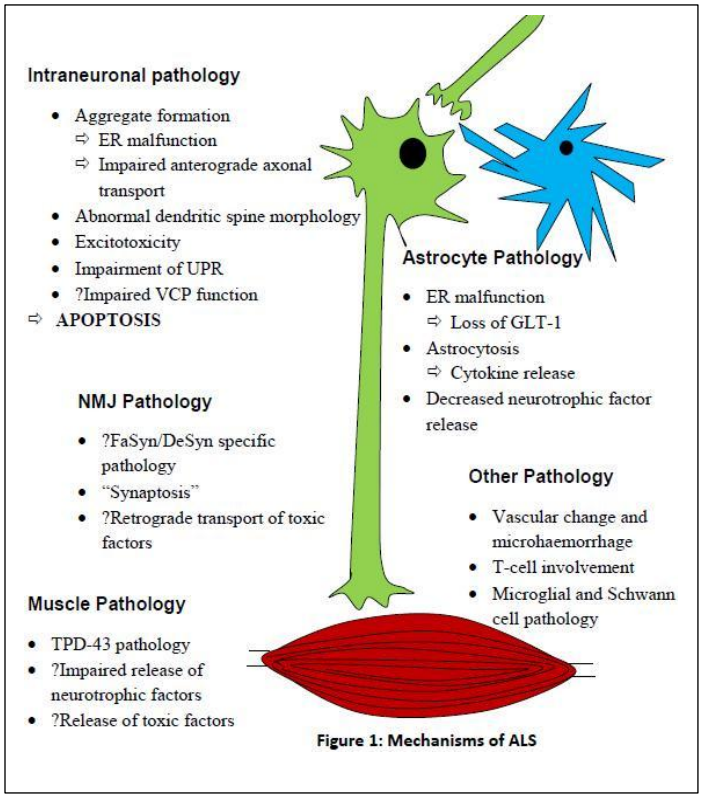

Figure 1: Mechanisms of ALS

There are numerous possible modalities of treatment currently being researched that target different etiologies of these motor neuron diseases. Potentially, these might yield an effective treatment or cure. The treatments include medications, immunizations, gene therapy and stem cell therapy and will be discussed in detail in the main body of the text.

The aim of this paper is to consider the pathogenesis of both diseases and compile a broad overview of the possible future therapies for ALS and SMA, their feasibility and effectiveness into one easily understood resource. This should give the reader a brief overview of a complex and rapidly changing field of research and provide references for future reading.

ALS overview

The precise pathophysiological mechanisms of ALS are largely unknown and there are many factors that may contribute to the overall disease mechanism (Figure 1). Research is complicated by the likelihood that different types of ALS; familial and sporadic – and even the different types of these – will have different causative mechanisms.

Familial ALS

Around 5% of ALS is known to be familial and inherited from the parents; however, the inherited mutation can be identified in only in 5-10% of these cases (Rosen et al. 1993). The most extensively researched locus is the SOD1 gene on chromosome 21. There are two main competing theories regarding the role of SOD1 in ALS. The first is that mutation to the superoxide dismutase gene causes a *loss of function* of the transcribed protein that increases the oxidative stress on tissues throughout the body and leads to excessive damage to the cell and DNA. However, it is unlikely that this alone is the cause of ALS because mice with homozygous knockout of the SOD1 gene show less neuronal degeneration than those with the mutated protein (Shefner et al. 1999).

An alternative theory is that SOD1 mutations cause the mutated protein to accumulate in cells which produces a toxic effect. This could be through formation of aggregate structures containing mutant SOD1 that appear histologically similar to the Lewy bodies found in Parkinson's disease. Thus this *gain in function* theory posits that causative mutations produce a new neurotoxic compound (Bruijn. 1998; Fridovich et al. 1969). This theory is currently the most popular and has the largest supporting evidence base.

Another gene that has been shown to be mutated in a significant number of ALS cases is the TAR-DNA binding protein (TARDBP) gene that encodes TAR-DNA binding protein-43 (TDP-43). Single studies have found 70 distinct point mutations in the TARDBP gene (Lee et al. 2009). TDP-43 mutations are present in some cases of both sporadic (Mackenzie et al. 2007) and familial ALS (Yokoseki et al. 2008; Cairns et al 2007). Indeed, it has been demonstrated that many sporadic and familial cases not due to a SOD1 mutation stain positive for TDP-43 aggregates whilst familial SOD1 cases were negative for the protein on immunohistochemical analysis (Mackenzie et al. 2007). This suggests a separate early mechanism of pathogenesis to that of familial SOD1 and that sporadic ALS and non-SOD1 familial ALS may have similar pathogenesis. SOD1 and non-SOD1 ALS disease pathways may converge to produce similar clinical and pathological findings. Initially, it was thought that effects of the TDP-43 mutation were confined to the motor system, but there is evidence that the gene is also involved in frontotemporal lobar degeneration (FTD) (Neumann. 2006). Little is known about this protein but it is postulated that it binds

DNA and RNA, altering the splicing and transcription of those molecules (Gong. 2000; Lee et al. 2009). It may also play a significant role in apoptosis and cell division (Sreedharan. 2008) and it has been suggested that TDP-43 acts as a scaffold for nuclear bodies that interact with SMN proteins (Mackenzie. 2007)

Mutations in the "*Fused in Sarcoma*"/"*Translated in Liposarcoma*" (FUS/TLS) gene have been demonstrated to be an important cause of familial ALS (Kwiatkowski Jr et al. 2009) that is negative for TDP-43 aggregates (Vance et al. 2009). Interestingly, mutations in this gene are also a cause of TDP-43 negative FTD (Neumann et al. 2009), thus demonstrating that, despite differing molecular causes of FUS and TDP-43 positive familial ALS, it is likely that their pathological mechanisms converge early on to produce similar phenotypes. It is possible that mutation of DNA motifs prevents localization of the FUS gene to the dendritic spine and nucleus which may be important for normal neuronal function and survival. This is because it has been demonstrated *in vitro* that FUS is transported to dendritic spines in hippocampal neurons in response to metabotropic glutamate receptor-5 (mGluR5) activation (Fujii et al. 2005), whilst neurons lacking FUS have decreased dendritic spine arborisation as well as abnormal spine morphology (Kwiatkowski Jr et al. 2009). Many mutations in FUS that result in ALS disrupt the non-classical PY nuclear localization signal (PY-NLS) pathway and furthermore, the age of ALS onset correlates with the degree of disruption of this nuclear localisation signal (Dormann et al. 2010). Therefore, it is likely that transport defects that result in either abnormal FUS aggregation or lack of FUS in crucial areas are very important in the pathogenesis of FUS positive ALS.

Recently, an exome sequencing study demonstrated that the gene encoding valosin containing protein (VCP) was mutated in some cases of familial ALS (Johnson et al. 2010). Mutations in this gene had previously been identified as a cause of inclusion body myopathy associated with Paget's disease of bone and frontotemporal dementia (IBMPFD). This is notable because of the common finding of TDP-43 inclusions in neurons in both ALS and IBMPFD (Weihl et al. 2008) and the common concomitance of ALS with frontotemporal dementia (Mackenzie et al. 2010). Although this study estimates that VCP mutation may account for just 1-2% of familial ALS cases based on their sample of patients, we hypothesize that VCP may be involved in ALS due to other mutations (see below).

The final gene that we shall discuss here is vesicle-associated membrane protein-associated protein B (VAPB). This is mutated in some cases of familial ALS (Nishimura et al. 2004). However, whilst this is a rare cause of ALS it might be very useful in illuminating pathological mechanisms (see below) (Dion et al. 2009).

Large-scale comparative genome analysis techniques permit comparison of the genomes of ALS sufferers and healthy subjects. Due to this technique, a number of small polymorphisms in specific genes have been associated with ALS (Berger and Cronin. 2008; Blauw and Veldin. 2008; Blauw and Cronin. 2008). A recent study used comparative genomic hybridization to find 11 small genomic variations in ALS patients that were not present in the normal population (Shoichet and Waibel. 2009). These may also provide a target for future research.

Interestingly, as with SMA, the inherited absence of functional SMN2 genes leads to a worse prognosis in ALS (Davies, et al. 2009).

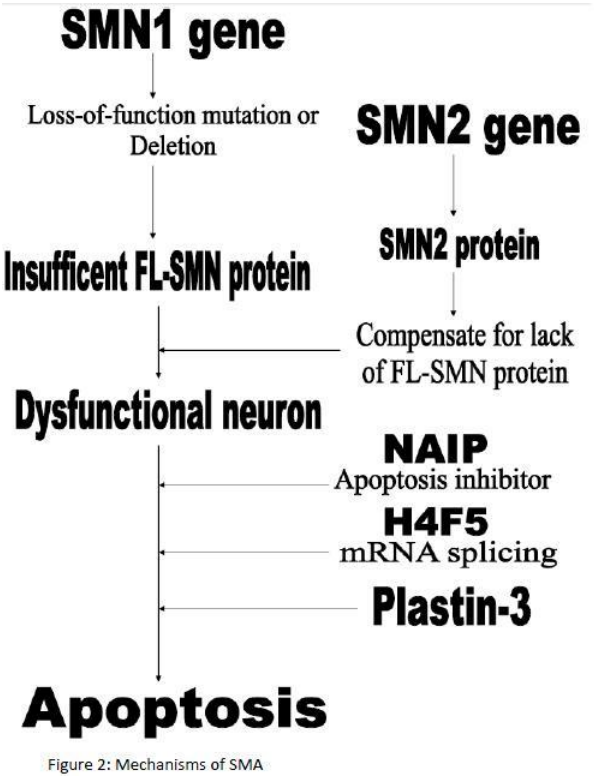

Figure 2: Mechanisms of SMA

Sporadic ALS

Sporadic cases of ALS account for over 90% of ALS cases and the majority of sporadic ALS cases are thought to be caused by a complex interplay of genetic and environmental factors (Schmidt et al. 2010). This means that the disease may arise from *de novo* mutations in a patient's DNA, rather than an inherited mutation. Numerous mechanisms for the development of sporadic ALS have been put forward, yet none have been conclusively proven.

Possible disease mechanisms for ALS

Neurodegenerative mechanisms

Glutamate excitotoxicity is the process by which elevated extra-cellular glutamate levels lead to neuron death. Astrocytes are the glial cells that normally express the GLT-1 transporter which transports and removes glutamate from the synaptic cleft. This transporter allows the astrocytes to regulate re-uptake of glutamate from the synaptic cleft and prevents the level of glutamate from becoming neurotoxic (Rothstein. 1996; Vanoni. 2004). High synaptic glutamate levels over-stimulate neuronal

calcium channels and the resulting calcium influx leads to the initiation of apoptosis. Elevated levels of glutamate have been found in the cerebrospinal fluid of patients with ALS (Spreux-Varoquaux. 2002). In addition, decreased GLT-1 levels have been demonstrated in some ALS patients (Levey et al. 1995). In rat models, the loss of the GLT-1 transporter in ventral horn cells directly correlates with the accumulation of SOD1 containing aggregates (Howland. 2002). It has also been shown that the knock-out of GLT-1 in normal rats resulted in neuro-degeneration and paralysis (Rothstein. 1996). Finally, it has been shown that intra-cellular aggregates, such as those of mutated SOD1 protein, can prevent the formation of the GLT-1 protein in the endoplasmic reticulum (Walker et al. 2009; Shibata and Hirano. 1994). Such studies provide compelling evidence for the role of glutamate toxicity in ALS.

The gene encoding VAPB has been shown to be mutated in some cases of ALS (Nishimura et al. 2004). Although this is likely to be a rare cause of ALS (Dion et al. 2009) it emphasises another aspect of the ALS pathogenesis. VAPB is a type 2 transmembrane protein (Nishimura et al. 1999) and has a role in intracellular membrane transport (Nishimura et al. 2004**),** as well as the unfolded protein response (UPR) pathway (Kanekura et al. 2009). The UPR is a mechanism by which the endoplasmic reticulum (ER) protects itself from the strain of having to process additional mis-folded protein during cell stress (Kanekura et al. 2009). The P56S mutation (which is the most extensively studied mutation of VAPB resulting in ALS) causes mis-folding of VAPB and hence a loss of function of the VAPB protein (Kanekura et al, 2006). Loss of function of the yeast homologue of VAPB results in increased susceptibility to ER stress (Suzuki et al. 2009). This implies that VAPB is required to augment the UPR. Other studies have shown that wild type VAPB negatively modulates the signal generated by the UPR signal generating protein, activating transcription factor-6 (ATF6). However, they demonstrated that P56S mutated VAPB displays increased ATF6 inhibition (Gkogkas et al. 2008). Therefore these studies provide good evidence that, at least in some cases of ALS, impairment of generation of the UPR signal may be involved in neurodegeneration. It is likely to be only part of the story however, as it has been shown that mice models of VAPB mutation develop TDP-43 pathology (Tudor et al. 2010). It is unclear how accurately this mouse models VAPB related ALS, as the mice do not develop motor symptoms (Tudor et al. 2010).

ER malfunction can cause disruption of GLT-1 formation, as mentioned above. It can also prevent the breakdown of abnormal protein in the cytoplasm. This can contribute to aggregate formation (Hirano and Shibata. 1994). Because of the numerous and diverse findings of ER pathology in ALS, further research is required to classify the nature of this in cases with differing genetic background and disease stage (Farg and Walker. 2009).

Johnson and colleagues recently demonstrated that VCP mutations play a causative role in some cases of familial ALS (Johnson et al. 2010). This accounts for relatively few cases of ALS but its involvement in ALS of other aetiology has not been excluded. This in turn may play a key role in the disease mechanism of a larger number of cases than the Johnson et al study shows. This is because of the finding that expression of the Wallerian degeneration slow (Wld^s) gene in SOD1 mice produces only a small increase in neuronal survival, and only during the early stages of disease (Fischer et al. 2005). For the Wld^s gene to produce the Wld^s phenotype of resistance to wallerian degeneration, the VCP binding region of the protein remain intact (Avery et al. 2008). We therefore hypothesise that in SOD1 mice there may be a lack of VCP, especially during later stages, that is required for the Wld^s phenotype to provide protection and ameliorate disease progress. This idea is backed up by the finding that Wld^s expression preferentially protects the soma and axon of neurons (Gillingwater et al. 2002) and has a much smaller protective effect on the NMJ because the NMJ is selectively vulnerable in SOD1 mice (Fischer et al. 2004).

Table 1: Management of ALS (adapted from Andersen et al. 2005)

Communicating the diagnosis and discussing the implications
Regular appointments and contact with multidisciplinary care
Neuroprotective treatment with Riluzole
Symptomatic treatment
Genetic counseling and analysis
Monitoring of respiratory function and non –invasive and invasive ventilation and treatment of dyspnoea
Assessment of nutritional status and providing nutritional support, including referral to a dietician and gastrostomy as indicated
Assessment of communication difficulties by a speech therapist
Palliative and end-of-life care

It is therefore plausible that disruption of VCP weakens the protective effect of Wld^s expression (which is already weak in the NMJ) in SOD1 mice. It may be that VCP binds to TDP-43 inclusions in ALS and is thus unable to function correctly (Johnson et al. 2010). This could contribute to development of the ALS phenotype. However, alternative explanations have been offered for the lack of effect of Wld^s expression in SOD1 mice such as loss of the protective phenotype with age (Gillingwater et al. 2002). Further work must be conducted as, with the finding of compensatory growth associated with ALS (Schaefer et al. 2005), one might expect the Wld^s phenotype to be recapitulated more completely at all ages in neuronal sub-populations that show new growth. This is because these neurons respond to Wld^s protein in the same way as neurons in juvenile mice

(Gillingwater et al. 2002). Also, it can be argued that the "dying back" phenotype of ALS (Fischer et al. 2004) might prevent protection from wallerian degeneration and prolong cell survival. However, because of the finding of fragmentation of the terminal bouton in ALS (Schaefer et al. 2005), the denervation of synapses that previously displayed compensatory growth in SOD1 mice should be lessened and should be clearly distinguishable in Wld^s/SOD1 mice from SOD1 mice by a phenotype reminiscent of juvenile synapse elimination in Wld^s/SOD1 mutants (Gillingwater et al. 2002). To test this hypothesis, VCP levels in SOD1 mice would need to be investigated. Study of *wobbler* mice may reveal early synaptic pathology as Johnson et al (2010) claim that these models of motor neuron disease have mutations in a mouse VCP homolog. It may follow that ALS progression can be slowed by treatment with inhibitors of wallerian degeneration such as FK866 (Hasmann and Schemainda. 2003; Sasaki et al. 2009).

Table 2: Symptomatic treatment (adapted from Andersen et al. 2005)

Sialorrhea (excessive secretion of salivia)	Treated with hyoscine, atropine drops, glycopyrrolate; portable mechanical home sunction device; botulinum toxin; irradiation of salivary glands
Bronchial secretions	Treated with portable home suction device and room humidifier; a mucolytics; nebulisers with saline and a beta-receptor antagonist, an anticholinergic bronchodilator, and/or furosemide in combination
Cramps	Cramps: physiotherapy; exercise; hydrotherapy; quinine sulphate
Spasticity	Physical therapy; hydrotherapy; antispastic drugs
Pain	Paracetamol and opiates
Depression	Antidepressants (such as SSRI, amitriptyline), counseling
Venous Thrombosis	Leg elevation and compression stockings. Anti-coagulation drugs are not recommended.

Degeneration in ALS is at least, partially due to apoptosis (Barbosa et al. 2010). This may be induced either by the damage to the cell incurred in the disease mechanism or ALS could directly modulate the mechanism of apoptosis. Mitochondria are intimately involved in the control of apoptosis. Mitochondrial dysfunction ultimately leads to caspase-mediated cell death and if this occurs in motor neurons, an ALS phenotype may result (Guégan and Przedborski S. 2003; Guegan et al. 2000). Mitochondrial dysfunction may be caused by calcium overload, as occurs in glutamate excitotoxicity, or by oxidative stress as mitochondria are the primary generator of reactive oxygen species (Barbeito et al. 2004). Caspase-mediated cell death is triggered by the release of cytochrome C from mitochondria. This activates caspases in the cytoplasm, which in turn initiate apoptosis. Mitochondrial dysfunction in ALS is wide spread, affecting the central nervous system (CNS) as well as skeletal muscle, blood lymphocytes and liver cells (Deschauer et al. 2005). This highlights the widespread damage that can be detected in ALS. Because of this research, the caspase cascade has become a target for pharmacological intervention as its inhibition could slow destruction of motor neurons and thus slow disease progression (Guégan et al. 2001).

Non-neuronal cell involvement

Experiments in which mutant SOD1 was only expressed in motor neurons resulted in a much lessened ALS phenotype (Lino et al. 2002; Pramatarova et al. 2001) which indicates that pathological change in more than one cell type is important for generation of the ALS phenotype. Motor neuron restricted SOD1 animals developed the disease much later than those expressing the mutant SOD1 ubiquitously, and progression was also slower (Jaarsma et al. 2008). Adding to this, it has been shown using chimeric mice models made of a mixture of normal and mutant SOD1 expressing cells, that having high levels of mutant SOD1 in most (Clement et al. 2003) or all (Yamanaka et al. 2008b) motor neurons is not sufficient for early onset disease. Therefore expression of mutant SOD1 in other cell types is important for disease initiation and disease onset is not cell autonomous.

The selective suppression of mutant SOD1 in motor neurons resulted in a slowed disease onset when applied at a young age, but did not affect disease progression when applied after disease onset (Ralph et al. 2005). Other experiments, utilizing Cre recombinase techniques to excise the mutant SOD1 gene from motor neurons also found similar results (Boillée et al. 2006a; Yamanaka et al. 2008a) These findings suggest that the expression of mutant SOD1 in motor neurons is important in disease onset and early progression, but not in later disease progression. Mutant SOD1 in other cell types must therefore be involved in disease progression after onset.

Astrocytes are cells which are found in close proximity to motor neurons. When levels of mutant SOD1 in astryocytes are reduced, disease progression is slowed and disease duration lengthened (Yamanaka et al. 2008a). Microglial activation is also affected and this suggests cross-talk between astrocytes and microglia. Astrocytes play a role in protecting motor neurons

from excitotoxic damage but when astrocytes express mutant SOD1, it has been found that the neurons are no longer protected (Van Damme et al. 2007). In addition, transplantation of astrocyte precursors delayed progression of disease after onset, highlighting a potential route for cell replacement therapies (Lepore et al. 2008). Astrocyte restricted expression of mutant SOD1 is not however sufficient to cause ALS (Gong. 2000).

Table 3

Ceftriaxone	Increases EAAT2/GLT1 activity, antioxidant
ONO-2506	Anti-inflammatory- Prevents reactive astrocytosis and COX2 inhibition; glutamate antagonism
Co-enzyme Q-10	Antioxidant; facilitates mitochondrial respiration
Memantine	N-methyl D-aspartate (NMDA) (glutamate) receptor antagonist- prevents glutamate excitotoxicity
MCI-186	Free radical scavenger; blocks mitochondrial transition pore; up regulates bcl-2 expression
Diaphragm pacing	Provide respiratory support and muscle training
Acrimoclomol	Heat shock protein inducer during cell stress. This stimulates normal cell repair pathways.
Antisense Oligonucleotide SOD1	Decrease production of SOD1 protein
Talampanal	α-amino-3hydroxy-5-methylisoxazole-4propionic acid (AMPA) (glutamate) receptor modulator. Thus decreases excitotoxicity.
TRO19622	Glutamate antagonist; anti-apoptotic
R+ pramipexol	Antioxidant

Astrocytes have also been implicated in other disruptions to the neuronal environment and neurotransmitter metabolism beyond that of glutamate. For example, there is evidence that cytokines released from astrocytes containing mutated SOD1 can lead to neuron death (Rothstein. 2009). However, neither the specific cytokines released nor the mechanisms behind their release are yet knowMicroglial cells have also been shown to be involved in ALS. When mutant SOD1 is excised from the microglia in rodents, disease progression becomes much slower, and overall survival is increased (Boillée et al. 2006a). In addition, when mutant SOD1-expressing microglial cells are replaced with normal cells via bone marrow transplantation, disease onset is not affected, but the progression is slowed (Beers et al. 2006). Therefore, it has been suggested that mutant SOD1 causes damage to the microglial cells, accelerating the progression of ALS (Ilieva et al. 2009*;* Boillée et al. 2006b). However, expression of the mutant SOD1 in motor neurons is required for disease onset (Ilieva et al. 2009; Boillée et al. 2006b).

Another non-neuronal cell implicated in ALS is the Schwann cell. These cells myelinate lower motor neurons to allow saltatory conduction. It was found that when dismutase-active mutant SOD1 was removed from Schwann cells, the late phase of the disease was accelerated (Lobsiger CS et al. 2009). It has therefore been proposed that increasing dismutase activity in Schwann cells improves the disease, suggesting that oxidative stress may play a role in the progression of ALS (Ilieva et al. 2009).

Finally, T lymphocytes have also been suggested to be involved in ALS, having a possible protective effect. Disease progression was found to be accelerated when T lymphocytes were prevented from entering the spinal cord of a mouse model of the disease (Beers et al. 2008; Chiu IM, et al. 2008).

Recent studies have highlighted the neuromuscular junction as a site of initiation of degeneration in ALS. This was first noted by Kennel et al using electrophysiological techniques. It was demonstrated that loss of neuromuscular transmission began as early as 40 days in SOD1 mice – before signs of axonal degeneration, as measured electrophysiologically (Kennel et al. 1996). A flurry of later studies confirmed this (Fischer et al. 2004; Schaefer et al. 2005) by the use of microscopy to show that end-plate denervation occurs before degeneration at the ventral root and spinal cord. Schaefer et al discovered that, at early stages of degeneration in SOD1 mice, three distinct subpopulations of NMJ could be identified: Those displaying degeneration; those displaying compensatory outgrowth; and those that appeared unaffected by SOD1 over expression at the time measured (Schaefer et al. 2005). These separate populations of “compensators” and “losers” could not be explained by the previous finding that fast-fatigable muscle fibers are more vulnerable to SOD1 over expression in mice than slow-fatigable muscle fibers (Frey et al. 2000), but might be due to the different degrees of vulnerability that Murray et al (Murray et al. 2008) demonstrated between FaSyn and DeSyn end-plates in SMA mouse models. These studies demonstrate that, in affected SOD1 mice muscles (and in one ALS patient), degeneration first manifests with end-plate denervation followed by centripetal

axonal degeneration and form the basis of the "dying back" hypothesis (Fischer et al. 2004).

There are several possible theories for why this should be the case. First, there could be a loss of transcription or anterograde axonal transport of some factor essential for survival of the NMJ specifically. Alternatively there could be a general disruption of the cellular machinery in the soma. This could result in those compartments of the cell that are furthest from the soma (i.e. the synapse) being the first to suffer. A compartmentalisation hypothesis has been proposed (Gillingwater and Ribchester. 2003) whereby different "compartments" (soma, axon and synapses) are affected in different ways by noxious or other stimuli (such as wallerian degeneration slow protein) because of their differing molecular and/or anatomical properties. Whilst these explanations might explain why NMJs in general appear especially susceptible to the disease process in ALS or mouse models thereof, they do not account for the finding of a compensatory subpopulation of neurons (Schaefer et al. 2005). If it is the case that muscular factors, such as FaSyn/DeSyn synapsing, determine neuronal susceptibility then further work must be carried out at the level of the muscle.

To attempt to address this, Wong and Martin (2010) used a skeletal muscle specific α-actin promoter to drive SOD1 expression solely in muscle. They found that this was sufficient to produce clinical and pathological phenotypes similar to other SOD1 mice. Interestingly, the time course of neurodegeneration more closely resembled a scaled-down timescale of human ALS (Wong Martin. 2010). The authors claimed that this was evidence of having produced a mouse model that more accurately modeled human ALS. However, there have been no human cases of ALS where SOD1 is expressed in a muscle specific fashion described. Muscle's role an ALS may be less than these studies (Wong Martin. 2010; Dobrowolny et al. 2008) imply, as reducing mutant SOD1 in muscle did not have an effect on the disease (Miller et al. 2006; Towne et al. 2008). This suggests either that muscle cells are not damaged by mutant SOD1 under normal disease conditions, or that muscle pathology does not significantly alter the course of neurodegeneration when neuron-specific pathology is also present. Furthermore, no benefit was seen when myostatin, an inhibitor of muscle growth, was inhibited in mice models of ALS (Holzbaur et al. 2006). Therefore muscle specific mouse models likely accentuate one aspect of a disease that has multiple foci, and may prove useful for dissecting the disease mechanism further. It would be interesting to examine these mice to see if they display the same neuronal subpopulations described above. This may distinguish between the effects of different NMJ subtypes (Frey et al. 2000; Murray et al. 2008) and of putative toxic factors, such as mitochondria transported retrograde from the NMJ (Fischer et al. 2004).

In addition to muscle, malfunction of the blood-brain barrier may precipitate or occur as a result of ALS. It has been observed in SOD1 mice that vascular changes in the blood-brain barrier may cause microhaemorrages. These may have no immediate symptoms, but over time, a build-up of neurotoxic products results in neuron malfunction. These vascular changes occur before neurodegeneration, which suggests that they may play a pivotal role in the early disease pathogenesis in ALS (Zhong et al. 2008). These studies all show that, whilst the most noticeable effects of ALS, as far as the patient is concerned, are neurodegenerative, it is over simplistic and incorrect to view this as a condition solely of the motor neurons (Figure 1). Therefore, further investigation of the non-neuronal and wider multisystem effects of ALS may present attractive therapeutic targets.

Table 4: Effects of treatment with valproate (Adapted from Swoboda et al. 2009)

Positive	Negative
Increased bone density	Hepatotoxicity due to low free carnitine levels
Increased maximum ulnar compound muscle action potential, indicating reinnervation of muscle	Motor unit number estimation showed no change
Slight clinical improvement in children aged over 2 years with Type II SMA	Weight gain (due to increase in fat mass)

SMA overview

SMA is an autosomal recessive disease that is relatively common in the human population. It describes a cluster of similar diseases that may manifest in infancy, childhood, or sometimes in adulthood. All forms of SMA are directly caused by different mutations in the same gene. This is termed the survival motor neuron (SMN) gene (Kesari. 2005). The classification system of the disease phenotype is straightforward. It is based on time of onset during life. SMA Type 1 (Werdnig-Hoffmann disease) presents within the first 3 months of life and currently is fatal within 2-3 years (Felina et al. 2007; Lunn and Wang. 2008). Type II SMA has its onset between 3 and 18 months: the affected infant/child may sit up but will never stand, and death is likely to occur within a few years of onset. Type III has its onset during adolescence and is associated with a normal lifespan. These patients usually will be able to walk but suffer from proximal muscle weakness (Lunn and Wang. 2008; Monani. 2005).

Genetic mechanisms

The SMN gene is located in the q11.2-13.3 region on chromosome 5 (Lefebvre. 1995; Lunn and Wang. 2008). Two forms exist side by side: SMN1 and SMN2, with the latter differing by 5 nucleotides. The SMN2 and SMN1 gene are the

same except that in place of an exonic splicing enhancer for exon 7 there is an exonic splicing silencer in SMN2 (Monani et al. 1999). This results in most, but not all, of the transcribed protein from SMN2 lacking exon 7. The main product of the SMN2 gene (without exon 7) quickly degrades in the cytoplasm and is thus has a 100 fold lower concentration in spinal cord of SMA patients compared with controls (Coovert et al. 1997). However, it is of importance that there can be several copies of the SMN2 gene present in humans (Cartegni and Krainer. 2002; Felina et al. 2007; Monani. 2005).

The complete SMN protein (i.e. containing exon 7) is termed full length SMN (FL-SMN) protein. The precise function of this has not yet been fully described but, as its name suggests, it is needed for the survival of lower motor neurons and their synaptic connections with upper motor neurons and with muscle. A number of studies have shown it holds an important role in RNA processing and splicing and is required for NMJ maintenance after birth (Felina et al. 2007; Monani 2005; Murray et al. 2008). As SMA is an autosomal recessive inherited condition, both copies of the SMN1 gene must be deleted or abnormal for the disease to develop. The most common mutation is a deletion involving exon 7 of the SMN1 gene (Felina et al. 2007). The severity of SMA is to a certain extent related to how well the remaining SMN 2 genes can make up for the loss of functional SMN 1 (Monani. 2005). In part, this is eon how many copies of the SMN2 gene are present in an individual's genome. If the resulting low levels of FL-SMN protein cannot support the needs of the motor neurons then disease occurs. Interestingly, if the person has 5 or more copies of SMN2, they typically show no SMA symptoms; the 5 copies appear to be able to make up for the loss of SMN1 (Figure 2; Felina et al. 2007).

The variable severity of SMA is not solely determined by the copy number of SMN2. There are other genes which can act as modifiers to the disease process (Figure 2). Two important genes, both also found in the 5q13 region of the genome, which act in this way, are termed: the NAIP (Neuronal-Apoptosis-Inhibitory-Protein) and H4F5 genes (Gendron and MacKenzie. 1999; Scharf et al. 1998)

It has been found that the NAIP gene is deleted in approximately 80% of SMA type I patients, but in very few individuals with types II or III (Monani. 2005). NAIP has been shown to be an inhibitor of apoptosis as it directly inhibits caspase 3, a pro-apoptotic protease involved in neuronal apoptosis. Therefore the absence or abnormality of this gene may allow apoptosis of motor neurons to become dysregulated. Thus, the deletion of both the SMN1 gene and the NAIP gene in the same person could cause a more severe form of the disease than if the NAIP gene was still present and functional.

H4F5 is also situated next to SMN1 and is deleted in more than 90% of SMA type I patients (Monani. 2005). It aids in the synthesis of snRNPs (small nuclear ribonucleoproteins), which form part of an organelle known as a spliceosome. This is a cytoplasmic complex responsible for mRNA splicing (Nilsen. 2003). The human genome contains a number of copies of H4F5 thus patients with fewer copy numbers develop a more severe disease phenotype.

Finally, a protein called plastin-3 is a newly discovered disease modifier that appears to be as involved in SMA pathogenesis as NAIP and H4F5. As of yet, there have been few studies on this protein, but it has been shown to be key in axonogenesis (Oprea et al. 2008). Thus, again, high levels of this gene's expression can go some way in counteracting the effects of the loss of SMN1 (Bowerman et al. 2009). This illustrates the extremely complex nature of SMA pathogenesis; disease phenotype is a product of a conglomerate of disease modifying genes that interact, in mostly unknown fashions with cellular changes induced by the SMN mutation.

Importantly however, although the FL-SMN protein can normally be found in the cytoplasm and nucleus of all cells in the human body with the exception of germ cells, the detrimental effect of loss of SMN1 is particularly noticeable in motor neurons. There are many competing theories for why this should be the case, but currently there is insufficient evidence to provide a satisfactory explanation. Why motor neurons should be particularly affected is an active area of research for SMA and other neurodegenerative diseases, including ALS.

Comparison

Clinically, SMA and ALS are fairly easy to distinguish from one another. The early onset of SMA and the restriction of the disease to the lower motor neuron clearly define the condition when compared with the adult presentation and upper motor neuron involvement of ALS. However, a confident clinical diagnosis is only the first step towards treatment. The cellular mechanisms that result in these pathologies are poorly understood and this makes formulation of effective treatments difficult.

Superficially, SMA seems relatively simple because a mutation of a single gene is the primary cause of the disease phenotype. However, the severity of the condition and therefore the prognosis depends upon a multitude of confounding genes. Furthermore, high copy number of the SMN2 gene can ablate the phenotype all together. This suggests that the crux of the conditions lies within the activity of SMN protein. Downstream interacting genes or gene products can complicate the picture by affecting the rate of apoptosis and neuron death and therefore determine the SMA disease type, rather than actually causing the disease. Consequentially, it may be extrapolated that treatments that attempt to replace either SMN1 protein or replace the neuron with a wild-type neuron will be successful in significantly prolonging lifespan in SMA patients whereas treatments that target apoptosis may only have limited potential in addition to more side effects.

Unlike SMA, ALS occurs as a result of disturbance of numerous dynamic relationships between cellular and genetic processes. Over time, this disturbance becomes greater and more widespread, perhaps due to accumulation of toxic proteins such as SOD1 and TPD-43 or triggering events, until it is sufficient to produce a phenotype later in life. Therefore, the mechanisms behind the ALS phenotype are more subtle and include satellite cell interactions as well as genetics. This complexity makes the mechanism harder to elicit but ultimately provides more

therapeutic targets. Genetic mutations to the SOD1 and TDP-43 gene loci are strongly associated with the ALS phenotype due to the cellular aggregates that interferes with GLT-1 production and results in excitotoxicity. The involvement of astrocytes and microglia demonstrates that ALS is a failure of more than the motor neuron itself. The protective effect of T-lymphocytes in the cerebrospinal fluid of ALS mice as well as the possible role of muscle pathology at even very early stages indicates that ALS may be a disease of more than the neurological system. Therefore, future ALS research must take a holistic approach and treatments designed to modulate the dynamic relationships between the neurons and their supporting glial cells. Because of the involvement of numerous pathways in the production of the ALS phenotype it is likely that effective treatment will require numerous pharmacotherapies to modulate the neuron-glia-body relationships. The development of a single therapy will require a greater understanding of the aetiological factors of sporadic ALS.

Current therapies for ALS

Currently, ALS is mainly treated by rehabilitation and symptomatic intervention. This is combined with pharmacological treatment, which attempts to slow disease progression.

Rehabilitation and symptomatic intervention

This focuses on psychological and physical support using a multidisciplinary team, highlighted by the European Federation of Neurological Societies (EFNS) guidelines (Table 1; Andersen et al. 2005). Initially, this involves discussion of diagnosis and the future implications of this. Effective communication and co-ordination between the hospital team, primary care team, palliative team and community services as well as follow-up are essential (Andersen et al. 2005).

Symptomatic treatment is important for maximizing patient comfort and independence (Table 2). Speech therapists and physiotherapists manage reduced function of peripheral and bulbar nerves. Mechanical aids such as walking sticks, wheel chairs and communication devices can help to reduce handicap. Cramps, spasticity and pain are mainly controlled by drugs and physiotherapy. Patients suffering from sialorrhoea are managed with hyoscine, portable mechanical home suction devices or irradiation of salivary glands. Increased bronchial secretions can be controlled with home portable devices and room humidifiers along with pharmacological intervention such as beta-adrenoceptor agonists or anticholinergic nebulisers. Venous thrombosis is a risk due to reduced mobility and is prevented by leg elevation and compression stockings. Many patients suffer from depression, anxiety and insomnia and require the use of antidepressants and counseling to ensure holistic care of the patient (Andersen et al. 2005). As with all genetic diseases, appropriate genetic counseling is very important.

In the later stages of disease, respiratory function should be monitored and non-invasive ventilator support can reduce the strain on the weakening respiratory muscles. Night time ventilation is commonly required due to the loss of tonic airway tone while sleeping. As bulbar palsy advances, percutaneous feeding tubes may also be necessary. There is some evidence that both early non-invasive ventilation and percutaneous endoscopic gastrostomy may prolong survival (Piepers et al. 2006; Mazzini et al. 1995). Patients with ALS require palliative care in the advanced stages of the disease which may include the use of sedative drugs (Andersen et al. 2005).

Pharmacological therapy

Currently, Riluzole is the only drug approved for the treatment of ALS and it was first developed 15 years ago to help delay onset of symptoms. The progression of ALS is slowed by early use of Riluzole and therefore it extends the life span of patients (Orrell. 2010). It is an expensive drug and there are concerns regarding its cost effectiveness.

Riluzole protects neurons from glutamate excitotoxicity (Aggarwal *and Cudkowicz.* 2008). The exact mechanism of action in slowing ALS progression however remains unknown. Analysis of efficacy in randomized controlled trials (Bensimmon et al. 1994; Lacomblez et al. 1996; Yanagisawa et al. 1997; Bensimmon et al. 2002; Traynor et al. 2006; Miller et al. 2007) indicates it can prolong median survival by 2-3 months. Riluzole appears to slow neural degeneration by blocking Tetrodotoxin (TTX)-sensitive Na^+ channels which are required for glutamate release and are associated with ALS (Miller et al. 2007; Shaw. 1999). Further understanding of this mechanism could result in the development of more advanced therapies enhancing this specific property of Riluzole that could slow or even halt the progression of ALS. Interestingly, other anti-excitotoxic drugs have failed to show the same efficacy (Aggarwal *and Cudkowicz.* 2008) implying that other properties of Riluzole may play important roles in the protective neuronal effect of the drug (Shaw. 1999). Since the discovery and use of Riluzole, many other drugs have shown potential promise for pharmacological invention, but so far no others have been translated into clinical use.

Current therapies for SMA

Similar to ALS, there is no curative therapy for SMA and current interventions are aimed at delaying disease progression and maintaining quality of life. A multidisciplinary team is required to provide comprehensive care. Genetic counseling, including screening family members and prenatal testing should also be discussed (Kostova et al. 2007). There are currently no drugs approved for the treatment SMA.

The treatment modalities can be divided into the areas that the disease affects. Muscle weakness, scoliosis and joint contractures are the major orthopedic challenges of SMA. Interventions include splinting and elastic arm slings, orthopedic chairs to reduce the frequency of joint contractures and neck braces to treat scoliosis, combined with rehabilitation which aims to maximize independence and mobility (Kostova et al. 2007) Similarly to ALS, as respiratory muscles weaken, a tracheostomy may be necessary to maintain airway stability (Hardart et al. 2002).Careful monitoring of nutritional intake is a major priority

for patients and occupational therapists aid in assessment of swallowing and feeding ability. A gastrostomy may be necessary as the disease progresses (Kostova et al. 2007).

Future therapies for ALS

Recent breakthroughs in understanding potential pathogenic mechanisms underlying ALS have highlighted numerous possible therapeutic approaches that are currently undergoing, or approaching, clinical trials (Table 3) (Aggarwal and *Cudkowic.* 2008). There are also many more therapeutic agents that are currently being tested on animal models or *in vitro* and a number of these are discussed below.

Human clinical trials

As well as the large body of research into riluzole, there have also been small-scale trials of antioxidant compounds in ALS. For example, vitamins C and E, selegiline, selenium, methionine and N-acetylcysteine, have been suggested as possible treatments (Orrell et al. 2007). The rationale for using these compounds is that they might compensate for the loss of superoxide dismutase's antioxidant properties. However, whilst they are well tolerated and do not cause serious adverse effects, administration of N-acetylcysteine showed no-significant improvement in survival for those whose disease started in the limbs (Louwerse et al. 1995).

Recently, the naturally occurring organic acid creatine has also been suggested as a potential treatment. However, the human clinical trials have produced mixed results (Pastula et al. 2010). Whilst creatine was well tolerated without any serious side effects, some studies have found that it did not significantly improve ALS symptoms or slow down disease progression and in some cases, breathing ability may have slightly worsened (Pastula et al. 2010).

Several neurotrophic factors have also been evaluated in clinical trials as part of the newly developing area of gene therapy. These trophic factors, such as Insulin like growth factor I (IGF-I), glial cell line-derived neurotrophic factor (GDNF) (Acsadi et al. 2002) and vascular endothelial growth factor (VEGF) (Storkebaum et al. 2005), are possible disease modifying therapies for ALS. They promote neuronal survival and have been shown to protect motor neurons from injury *in vivo* and *in vitro* (Shaw. 1999).

VEGF has been shown to be neuroprotective in patients with sporadic ALS (Zavalishin et al. 2008). It is hypothesized that it can prevent or minimize putative ischaemic damage (Oosthuyse et al. 2001; Zhong et al. 2008). However, a recent in-vitro study demonstrates that the neuroprotective mechanism of VEGF in ALS is due to VEGF causing a glutamate channel in motor neurons to become impermeable to calcium and thus not susceptible to excitotoxicity (Van Damme. 2009). Although VEGF's mechanism of action in ALS is poorly understood, treatments involving this protein stand out from other treatments utilizing trophic factors because of the number of studies demonstrating efficacy and high tolerability (Zavalishin et al. 2008; Storkebaum et al. 2005).

Recombinant human insulin-like growth factor (rhIGF-1) is a genetically engineered human protein that was expected to increase the survival of motor neurons that degenerate in ALS (Lai et al. 1997). Indeed, it produced a positive effect in slowing the progression of ALS but the result was not replicated in a second trial (Mitchell et al. 2007). When given subcutaneously, two other small randomized control trials have shown a slight significant benefit in delaying disease progression (Lai et al. 1997).

The route of administration for these neurotrophic factors has been, to date, via subcutaneous injections. There is a possibility that this route of administration means that the compounds are not reaching motor neurons in sufficient amounts to exert a maximal effect. It has been suggested that direct intra-thecal administration via an implanted infusion pump is a possible solution (Shaw. 1999).

Experimental models

The SOD1 mouse has been used extensively to study potential therapeutic agents for human ALS. However, although many of the drugs trialed have been found to be effective in the mouse models, very few of them have been able to produce a therapeutic benefit in humans. Successful treatments in the mouse model are may only be effective in patients with the familial SOD1 form of the disease (Dal Bello-Haas et al. 2008) which only accounts for a very small proportion of ALS patients. Currently, most studies have failed to acknowledge or account for this during trial design.

One agent investigated with the SOD1 mouse, arimoclomol, has been shown to be effective in terms of delaying disease progression and extending lifespan. The drug has also been tested in patients for safety and concluded it was well tolerated. An efficacy study in humans has been planned (Kalmar et al. 2008; Cudkowicz et al. 2008; Kieran et al. 2004). Furthermore, ceftriaxone has also been proven to be beneficial in the mouse model by increasing GLT1 expression, which leads to an increase in the clearance of glutamate, helping to decrease the progression of the disease (Rothstein et al. 2005; Brown. 2005) Other compounds, such as antisense oligonucleotides, reduced SOD1 levels throughout the mouse's brain and spinal cord (Traynor et al. 2006).

Alongside the potential therapeutic treatments detailed above, there is also a possible prophylactic treatment for ALS using glatiramer acetate (a new drug used for multiple sclerosis). This drug was found to prolong survival in SOD1 mice models (Angelov et al. 2003) and it has been suggested that such a treatment might inspire future use of vaccination to prevent ALS. However, results obtained to date indicate that it is likely to work by slowing the disease process rather than inhibiting onset (Angelov et al. 2003). One antioxidant compound, vitamin E, has been found to be beneficial in SOD1 mice where it delayed the symptom onset and reduced brainstem pathology as measured histopathologically and using magnetic resonance imaging (Chang et al. 2008; Bucher et al. 2009). The utility of this in

humans is however likely small, as discussed above, especially if the compound must be administered before symptom onset to be of use. Gabapentin has been shown to be beneficial by reducing glutamate excitotoxicity. However, rather than delaying disease onset, it caused extended survival once the disease had already manifested (Gurney et al 1996).

Through survival-promoting effects on motor neurons, ciliary neurotrophic factor (CNTF) has been shown to slow disease progression and improve muscle strength in the progressive motor neuronopathy mouse model of motor neuron disease (Sendtner et al. 1992). In human clinical trials in which CNTF treatment was used however, results did not show any significant effect in slowing the progression of ALS and adverse effects were observed at high concentrations (Bongioanni et al. 2004).

Clinical studies of IGF-1 administration in humans have led to disappointing results. However, as mentioned above, the effect may be dependent on the modality of administration. Kaspar et al. (2002) demonstrated that retrograde transport of adeno-associated viruses (AAV) could allow for increased production of proteins encoded by viral DNA (in this case Bcl-2) in presynaptic cortical neurons. Using this model they next demonstrated that intramuscular injection of SOD1 mice with AAV vectors carrying IGF-1 caused increased IGF-1 production in the spinal cord and improve clinical phenotype and lifespan (Kaspar et al. 2003). Crucially, this benefit was seen even if the AAV was administered post-clinical onset. Even though only a very small number of viral particles made it into the spinal cord and these might have found residence in afferent neurons or glia this was sufficient to produce enough IGF-1 to be clinically relevant (Raoul and Aebischer. 2004). Therefore further work to improve localization and maximize retrograde transport would be very helpful.

Another gene that modifies disease progression is cardiotrophin-1 (CD-1). This muscle derived protein is a member of the interleukin-6 family of cytokines and improves the clinical and neuropathological phenotype of pmn mice (Bordet et al. 1999). In SOD1 mice intramuscular injection of CD-1 carrying AAVs produced a modest increase in survival time and improved motor function (Bordet et al. 2001). This effect may be due to CD-1's effect on BDNF, GDNF, CNTF and leukemia inhibitory factor (LIF) sensitive motor neurons (Oppenheim et al. 2001).Other investigations have been carried out by cross breeding SOD 1 mice in order to see the effects of changes in gene expression on murine ALS (Shaw. 1999). For example, concomitant over-expression of SOD1 with Bcl-2, a protein that inhibits apoptosis, slowed down the onset of motor neuron degeneration in mice with the mutant enzyme (Kostic et al. 1997).

Another important example is a study of the effects of tissue specific up-regulation of GDNF in SOD1 mice. Whilst up-regulation of GDNF in astrocytes driven by a glial fibrillary acidic protein promoter did not effect histopathological or clinical measures, when expression was driven by a muscle-specific promoter significant changes were seen (Li et al. 2006). Muscle specific GDNF extended life expectancy and improved locomotor performance. Furthermore histopathological measures were greatly improved in these mice (Li et al. 2006). These studies, show a trend for increased efficacy of neuroprotective proteins when expressed in muscle and when examined in light of Wong and Martin (2010)'s study, provide a strong case for increased study of pharmacological therapies targeted to muscle.

By observing animal and cellular models, our knowledge of the cellular mechanisms of ALS is likely to be clarified and expanded in the future. This will enable the development of more effective neuroprotective treatment for patients, with future treatment being likely to involve a "cocktail" of neuroprotective compounds interfering with several neuronal injury pathways, inducing a synergistic response.

Future therapies for SMA

There are a number of promising pharmacological treatments for SMA that are currently being trialed. Many of the drugs are still at an early stage of human clinical trials, but some are showing modest yet significant results.

Riluzole

As detailed above, Riluzole is currently the only drug approved as a therapeutic for ALS (Orrell. 2010). It is thought to be neuroprotective through its actions on reducing glutamate release and therefore excitotoxicity (Bosboom et al. 2009). Due to its effectiveness in ALS there has been some research into its action in SMA. For example, Russman et al. (2003) carried out a randomized controlled trial, comparing riluzole treatment with a placebo. The trial was carried out on SMA type 1 patients, who have a normal life-expectancy of 24 months. The study found that although the three members of the placebo group died within 24 months, three out of the seven riluzole-treated group were still alive at 30, 48 and 64 months. This was only a small study, but it did show some promise, especially as no adverse effects of treatment were reported. However glutamate excitotoxicity is not currently thought to represent a major pathogenic mechanism underlying SMA. Therefore, in SMA it might be playing a role in decreasing strain on axonal transport systems that may become impaired (Monani. 2005).

Valproic Acid

Valproic acid is a histone deacetylase (HDAC) inhibitor that has been considered for the treatment of SMA. Histone proteins are encased in DNA within the chromosome and are responsible for determining the ease by which transcription machinery can reach the DNA. Histones are removed by the histone deacetylase enzyme. Blocking such enzymes means that the SMN2 gene is more accessible to transcription machinery and therefore transcribed more freely. With an increase in the expression of the SMN2 gene, this can compensate for the loss of the SMN1 gene by increasing production of FL-SMN and therefore potentially reduce the severity of SMA (Mercuri et al. 2007; Monani. 2005).

An open label, 6 month study of 42 patients (Table 4; Swoboda et al. 2009), found a slight clinical improvement and increased muscle innervation in children over 2 years with type-

II SMA. These signs indicated re-innervation of muscle by 'sprouting' and an increase in bone density. Changes in pulmonary function were observed but not considered statistically significant. The benefits of valproate treatment were therefore significant, but small. However, there was a high risk of toxicity due to altered carnitine metabolism. Therefore the net gain in treatment would have to be carefully considered for different groups of patients before this drug becomes routinely prescribed.

Hydroxyurea

Hydroxyurea is also a HDAC inhibitor. It has been shown to increase SMN2 expression in-vitro in SMA type II and III (Bosboom et al. 2009). Liang et al investigated the effect of varying hydroxyurea concentrations on type II and III SMA cells and in patients too. They treated lymphoid and fibroblast cell lines with the HDAC inhibitor and found that SMN gene expression was enhanced. Patients (n=33) were treated for eight weeks with different doses of hydroxyurea, and then followed up for another eight weeks. Liang et al. (2008) found that muscle strength scores at 4 weeks were slightly increased, as were the expression of the SMN gene at 8 weeks in the moderate dose (30 mg/kg/day) subgroup. The adverse effects of hydroxyurea, such as bone marrow suppression, were also recorded at 30 and 40 mg/kg/day and therefore Liang et al suggested that future trials of this treatment should use a dose of less than 30 mg/kg/day (Liang et al. 2008).

Phenylbutyrate

Phenylbutyrate, another HDAC inhibitor, functions by the same mechanism as valproic acid and so is thought to increase the expression of the SMN2 gene and compensate for the absence of SMN1.

Mercuri et al. conducted a case-control study in 2007 to identify its efficacy (Mercuri et al. 2007). Though 40% of the subjects on phenylbutyrate showed slight improvements in functional tests, it was not significant when compared to the placebo group. It was therefore concluded that phenylbutyrate had no significant effect on the gross motor function of SMA patients and has since been discarded as a possible treatment. This is particularly interesting considering that valproic acid operates by the same mechanism and displays significant improvement.

Trichostatin A

Another HDAC inhibitor that currently shows promise in treating mouse models of SMA is Trichostatin A (Codd et al. 2009; Vanhaecke et al. 2004). This drug has been shown to increase SMN2 gene expression in mice, and is associated with improved survival and motor control (Narver et al. 2008; Avila et al. 2007). It is thus a promising avenue of research and trials continue.

Additionally, further study of Trichostatin A's mechanism of action may help delineate the pathophysiology of SMA. It has been shown in SMA mice models, that treatment with Trichostatin A increases muscle size and levels of cholinacetyltransferase (ChAT) in the spinal cord (Avila et al. 2007). Both of these effects are seen specifically when muscle size and condition is improved, as opposed to neuronal improvement (Lowrie et al. 1987). This offers some evidence that SMA may be a disease more of muscle than is currently supposed and hints at a parallel disease mechanism to ALS (Wong and Martin. 2010)

Thyrothrophin-releasing hormone

Thyrotropin-releasing hormone (TRH) is a peptide produced by the hypothalamus that is classically thought to stimulate the pituitary to release thyroid stimulating hormone (TSH). TRH receptors have been found in anterior horn cells (Tzeng. 2000) and so it has been suggested that increased levels of TRH may increase neurone cell firing (Bosboom et al. 2009). A small randomized controlled trial in 9 patients with SMA type II or III was carried out (Tzeng. 2000). Treatment resulted in significant small increases in muscle strength and adverse effects were quickly resolved.

Albuterol

The beta-2 receptor agonist albuterol has also been considered as a possible treatment for SMA and particularly SMA Type II. The exact mechanism by which this drug could produce a clinical improvement is not fully understood but it is currently hypothesized that it may reduce wasting in denervated muscle or supports the repair of damaged fibers (Pane et al. 2008). Pane et al. (2008) treated 23 children with the drug and found significant improvements in strength and stamina of the children after 6-12 months. It was concluded that further studies would be required, preferably a randomized, double-blind, case-controlled study (Pane. 2008).

Gene Therapy

SMA is a monogenic disorder and so the re-introduction of a wild-type SMN1 gene into motor neurons may prevent disease progression. This makes it a promising target for gene therapy. Neurons are permanent cells and so may only require a single treatment compared with other genetic conditions such as cystic fibrosis where the short life-span of treated cells necessitates multiple courses of treatment (Gill et al. 1997). A large difficulty currently being faced is finding appropriate receptors on target cells to allow vector binding.

Replacement of the SMN gene can be achieved through a viral vector in a number of animal models. This can prevent neurodegeneration and even promote motor unit rescue if the animal is infected early enough (Foust. 2010).

Another approach to gene therapy in SMA is to correct the splicing of functional SMN2 genes to produce FL-SMA protein. Using plasmids as vectors, introduced RNA that optimizes *trans*-splicing has been shown to increase intron 7 inclusion. This technique was found to almost double the average lifespan of severe phenotype mouse pups (Coady and Lorson. 2010). It is also possible to introduce bifunctional RNA that silences a splice codon and therefore causes the RNA to retain exon 7, resulting in formation of a functional protein. This has been demonstrated in fibroblasts from human SMA patients. The

RNA was then introduced via a plasmid and the cells showed increased levels of the SMN protein after only 48 hours. In neonatal mice the introduction of bifunctional RNA into the spinal column resulted in increased SMN protein and also increased lifespan. (Baughan et al. 2009).

Neuronal apoptosis is also a target for gene therapy in SMA. Over expression of apoptosis inhibiting enzymes could protect the neuron from apoptosis. For instance, SMA type III mice modified to have increased Bcl-2 XL expression demonstrated a 50% increase in survival and an improved phenotype (Tsai et al. 2008). However, this over-expression was induced by genetic modification prior to birth, not by treatment afterwards. For use as a treatment in humans, a vector would have to be created that could carry genetic material to induce increased Bcl-2 XL expression after birth. Bcl-2 XL is an important regulator of cell growth and so treatment would need to be very specific to avoid tumorigenesis.

Stem cell therapy for ALS and SMA

Stem cell therapy for motor neuron disease promises a cure for both ALS and SMA, however the technical difficulties involved in successful re-innervation of motor units may impede its use in the foreseeable future. For both ALS and SMA the basic methods of culturing and administering stem cells are similar. However, each disease presents unique difficulties.

Harvesting stem cells and stimulating differentiation and growth to replace diseased neurons is a multi-step process. Once harvested from embryos cultured from IVF techniques, mouse pluripotent embryonic stem cells can be manipulated to exhibit neuronal characteristics and even form NMJs (Deshpande et al. 2006). It has also been demonstrated that the myogenic stem cells that migrate from the bone marrow to reside between the basal lamina and the sarcolemma may provide an in-vivo source of stem cells (McKinney-Freeman et al. 2002). Once isolated, these cells can be manipulated, tested and allowed to proliferate and are theoretically immortal cell lines. Although these cells are classically committed to a myogenic cell line, they can be forced to defy germ layer commitment and be stimulated to become neuron-like cells (Wu et al. 2010).

The choice of stem cell donor presents a dilemma (Abdelkrim et al. 2009). The use of patient's own cells would have a lower likelihood of graft rejection but would require gene therapy to correct the mutation genes. On the other hand however, a healthy donor stem cell would need to be matched for tissue type and risk graft rejection. Although these techniques are possible *in vitro*, difficulties arise when carried out *in vivo*. Cells that are treated to differentiate into neuron-like cells are highly dependent on SMN to prevent apoptosis and showed a diminished viability of 40% at 48hrs (Kerr et al. 2000). This effect is specific to neuron-like cells and SMN mutant undifferentiated cells formed colonies similar to wild type controls. Due to this, the possibility of using patient stem cells to regenerate lost neurons seems unlikely as injected stem cells could proliferate but once they began to differentiate, they would undergo apoptosis. Moreover, only 0.6% of all transplanted neurons innervated the ventral root and none innervated the motor unit in SMA mice (Henderson et al. 1994), even though this was carried out in simple rodent models. However, a preclinical safety study into the technique of neural progenitor cell grafts by injection has indicated that the technique is safe (Riley et al. 2009). Another concern is that, due to the length of human motor neurons, the axons would take months or years to grow to the required length (Papadeas and Maragakis. 2009).

Research into the use of stem cells to replace muscle tissue in SMA has shown some promise (Nicole et al. 2003). However, muscle tissue and neuronal tissues are not immunoprivileged sites and their antigens are exposed to the scrutiny of the immune system (Chidgey et al. 2008). Therefore, the issue of graft rejection remains. Embryonic stem cells that are yet to develop adult antigens could be used. It is however, possible that adult immune system would reject the embryonic surface antigens or the cells would be susceptible to natural killer cell attack (Drukker and Benvenisty. 2004). They may also result in teratoma formation in the immunocompromised (Ilancheran et al. 2007). Injection of some therapeutic stem cells into the thalamus has been suggested as this would introduce central tolerance of the graft. In addition, the use of tolerance-inducing dendritic cells could induce peripheral tolerance (Chidgey et al. 2008). It has been hypothesized that the major histocompatibility complex (MHC) complex genes could be knocked out in transplanted tissues and this would help cloak the graft from the patient's immune system (Yang and Lui. 2008). Even this approach is not without its difficulties as this will likely lead to cell death by natural killer cells (McNerney et al. 2006). The problems of graft tolerance are vast and must be fully understood and resolved before the use of non-autologous stem cell grafts to treat SMA becomes a reality.

Another source of stem cells is the pluripotent cells found in the adult bone marrow or, for SMA research, the bottom of the spinal cord. They vary in differentiation and their function is to replace damaged cells in their associated tissue. These stem cells can be induced to differentiate into motor neurons (Papadeas and Maragakis. 2009). Spinal Muscular Atrophy with respiratory distress type 1(SMARD1) mice treated with neuron transplants and drugs to promote axonal growth showed less degeneration in postural tone than untreated controls (Corti et al. 2009). Most SMA stem cell research has been carried out in basic animal models and studies show that the transplanted motor neurons can survive for months (Deshpande et al. 2006). However, it is very difficult to induce the two ends of the impulse chain to connect in SMA models and therefore the implanted stem cells serve no physiological function.

In ALS, there are two main strategies for stem cell replacement: replacing motor neurons and replacing non-neuronal cells (Wichterle et al. 2002; Corti et al. 2009). Motor neurons have been developed from mouse embryonic stem cells and it has also been demonstrated in rodents that stem cells may rescue mice that have had their motor neurons removed or damaged (Deshpande et al. 2006; Gao et al. 2005). These studies however, do not use ALS mice models and they rely on a single insult to the motor neurons before using stem cells to rescue them. They also only attempt to re-grow lower motor neurons

and therefore do not address the damage caused to upper motor neurons in ALS. Moreover, the main problem currently is that the size of motor neuron that would have to be grown in humans is much larger than that for mice and would therefore take months or years to grow (Corti et al. 2009). Until these problems are overcome, replacing motor neurons will not become a viable treatment option.

Replacing damaged non-neuronal cells with stem cell derived astrocytes or microglial cells is the other major strategy for stem cell therapies in ALS (Corti et al. 2009). In rodent models of ALS, death occurs due to type II respiratory failure (Tankersley et al. 2007). Therefore in an attempt to extend the lifespan of SOD1 rats, astrocyte precursor cells were injected into the ventral cervical spinal cord (where the phrenic nerve exits the spinal cord) which delayed motor function decline and also improved survival (Lepore et al. 2008). These cells were also found to survive *in vivo* in this model of ALS and differentiated into astrocytes.

Suzuki et al. investigated the introduction of neural progenitor stem cells into SOD1 rat spinal cords. They replicated and migrated to areas of motor neuron degeneration where they secrete glial cell derived neurotrophic factor (GDNF) which is known to protect motor neurons from a range of insults. This succeeded in protecting motor neurons but failed in preventing denervation of muscle end-plates and thus in ameliorating disease progress (Suzuki et al. 2007). To address this, they then introduced stem cells secreting GDNF directly into muscles which increased innervation and also delayed degeneration in the spinal cord (Suzuki et al. 2008). However, caution must be taken when introducing foreign cells into any tissue, due to the risk of tumorigenesis (Amariglio et al. 2009).

Discussion

Producing effective treatments for ALS and SMA has proven to be very difficult. In both diseases, the precise mechanism of motor neuron degeneration has proven to be elusive and because of this the main treatment has been of a symptomatic, physiotherapeutic and palliative nature. Riluzole remains an expensive and ineffective treatment option for ALS (Miller et al. 2007) and possibly SMA (Russman et al. 2003). For effective treatments to be created a greater understanding of the disease mechanisms must be achieved. Once this is accomplished, it will be possible to use rational drug design to target specific stages in disease progression of ALS and SMA thus slowing or stopping their progress.

Currently, it appears that the most realistic strategy for developing therapeutics for both diseases is to focus on supportive treatments. The use of neurotrophic factors can prolong survival and hinder neurodegeneration. In ALS, where significant glial cell pathology is evident, neurotrophic support may compensate for the reduction in astrocyte function. In both diseases there has been an increased focus on the involvement of muscle and the NMJ. Because of the ubiquitous expression patterns of most proteins associated with both ALS and SMA it is likely that multiple systems contribute towards the pathogenesis of ALS and SMA. These have begun to be investigated but further work must be carried out to investigate how non-neuronal systems might impact on neuronal survival.

The process of translating treatments that ameliorate symptoms in mouse models of ALS into the human model has been especially difficult. Many researchers now criticize the current models of ALS, especially the SOD1 mouse (Benatar. 2007). Others have suggested that more stringent experimental design and statistical analysis is required (Scott et al. 2008). However, in SMA the main obstruction to the development of effective treatments has been the high cost to benefit ratio of these treatments. The most commonly researched avenue of treatment – the use of HDAC inhibition – causes a relatively non-selective up-regulation of protein synthesis. Whilst it may be possible to target these drugs towards specific tissues, it is unlikely, in our opinion, that this treatment strategy will be able to achieve single gene selectivity in the near future. Therefore, we believe that for SMA, gene therapy is a more realistic curative treatment option. Problems regarding the safety of adeno-associated virus targeting the CNS may be bypassed by the use of intramuscular injections and subsequent retrograde transport.

The use of such technology in ALS will not be able to produce a curative therapy in the near future. A lack of understanding of the causative factors in sporadic ALS primarily hampers this. It is unclear if findings in the SOD1 mouse truly represent the majority of ALS cases. The recent development of a TDP-43 pathology mouse model might allow for confirmation or refutation of many of these findings when applied to sporadic ALS (Wils et al. 2010). However, these mice are not an exact model of human ALS as they do not rely on mutations to the TDP-43 gene, but rather on its' over expression to produce pathology. The development of an optimal therapy will ultimately require much more research into the basic science. This should especially focus on understanding the causative mechanisms of sporadic ALS. A novel approach, recently used by O'Dushlaine et al, provided insight into the genetic contribution to molecular mechanisms of major psychiatric illness, another pathology where the causative mechanisms are far from clear. The team analysed the contribution of different gene alleles in over 200 molecular pathways using a single-nucleotide polymorphism ratio test to ascertain what molecular pathways were involved in the diseases they were studying (O'Dushlaine et al. 2010). Such an approach could be used to determine which pathways are involved in ALS. This has advantages over genome-wide association studies as it raises the power of studies with small sample sizes if multiple alleles of different genes in the same pathway contribute to pathogenesis and it also is resistant to variation in linkage disequilibrium.

The key factor that links both ALS and SMA is neurodegeneration. Whilst this end-point appears to be arrived at by very different mechanisms, both conditions can be partially rescued by up-regulation of the anti-apoptotic protein Bcl-2 (Kostic et al. 1997; Tsai et al. 2008). This non-specific inhibition of apoptosis is clearly not feasible for use in humans because of the risk of tumorigenesis. However these findings provide hope that, at some point, a universal agent that protects against motor

neuron degeneration could be created. Therefore, it is essential that we reach a greater understanding of what makes motor neurons susceptible to the changes that occur in these diseases.

Conclusion

In this review we have discussed in detail what is known of the pathogenic mechanisms of two motor neuron diseases that present primarily with motor dysfunction and progress to paralysis. The genetic contribution to SMA is very well characterized; the downstream molecular mechanisms of neurodegeneration are not. In ALS, the reverse is almost true. The mechanisms that lead to cell death are well understood, although the reasons for motor neuron susceptibility or environmental triggers of the condition are not well described. Causative factors have only been described in a small number of familial ALS patients and very little is known regarding the causes of sporadic ALS.

SMA is caused as a result of loss-of-function mutations or deletions of the SMN1 gene. As a consequence of this it is perhaps not surprising that the most effective treatments – HDAC inhibitors and viral integration of functional SMN1 – have focused on ameliorating this loss rather than attempting to block the downstream effects of fl-SMN protein insufficiency. It thus follows that the use of gene therapy in this field may be very effective in humans. ALS however, appears to be, at least in familial cases, due to the production of neurotoxic compounds. Therefore, the most effective treatment strategies in mice have attempted to block specific toxic effects such as glutamate excitotoxicity. In the future, the use of stem cells in the treatment of both conditions will likely be beneficial. In our opinion, this will not be via a neuron replacement strategy, but through their use as vectors to deliver neurotrophic compounds or by supporting failing non-neuronal cell types.

Acknowledgements

We thank Prof Thomas Gillingwater for his assistance and direction throughout this project and writing of this paper. Lewis Hou also helped with critical reading of the paper.

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