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Neuroprotective agents target molecular mechanisms of disease in ALS

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Amyotrophic lateral sclerosis (ALS) is a debilitating disease characterized by progressive loss of voluntary motor neurons leading to muscle atrophy, weight loss and respiratory failure. Evidence suggests that inflammation, oxidative stress, mitochondrial dysfunction, apoptosis, glutamate excitotoxicity and proteasomal dysfunction are all responsible for ALS pathogenesis. We review neuroprotective agents with the ability to reduce ALS-related bodyweight loss, summarize the various therapies tested on animal models targeting the proposed molecular mechanisms, compare their effects on bodyweight loss, muscle damage, disease onset, duration and survival, and analyze their structure–activity relationships, with the overall goal of creating a screening strategy for further clinical application.

Introduction

Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease, was first described in 1869. The prevalence of ALS is ~30 000 in the USA, with a slightly higher incidence in men. About 10% of ALS cases are familial (fALS) [1] and the most common mutation known to cause fALS is C9orf72, a hexanucleotide repeat expansion [2]. The second most common mutation is found in the copper/zinc superoxide dismutase (SOD1) gene, resulting in a toxic gain of function [1]. About 90% of ALS cases are sporadic (sALS) and unassociated with any known genetic mutations. Considering the relatively high incidence and severity of ALS, there is a severe lack of effective clinical treatment. Riluzole, the only FDA-approved treatment, prolongs patient life by only three months. Thus, curative therapies are urgently needed [3].

Weight loss and muscle atrophy in patients and animal models of ALS

Weight loss is ubiquitous among ALS patients [4–6] and can arise through loss of muscle mass [6,7]. Muscle ultrasonography reveals marked abnormalities including diminished thickness, increased echo intensity and fasciculation. Muscle thickness correlates with bodyweight, function and grip strength [8]. In 50–81% of patients with ALS the presenting sign is dysphagia [9,10], which results from oropharyngeal muscle dysfunction [11] and affects quality of life in nearly all patients. Dysphagia can lead to weight loss via decreased food intake and malnutrition [9,10].

Hypermetabolism is a generalized metabolic dysfunction also present in patients with ALS [12–14], which, combined with negative energy balance, leads to bodyweight loss [5,9]. The mechanisms underlying hypermetabolism in ALS remain unknown, but growing evidence indicates that mitochondrial defects (mainly muscular in origin) inherent to the disease, not only in motor neurons but also in skeletal muscle [12], drive hypermetabolism [15,16].

A loss of bodyweight >10% or a body mass index (BMI) <18.5 kg/m^2 in ALS patients are negative predictors of survival [17]. Significant evidence suggests that low BMI and malnutrition can increase mortality of ALS patients sevenfold [18–20]. Survival outcomes in patients with BMI 30–35 were better than those with a BMI <30 or >35 [21]. Bodyweight loss in the first two years after diagnosis significantly correlates with shorter survival and faster

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progression [22]. However, a study on athletes with ALS reported that slimness might be an early manifestation of ALS rather than a risk factor [23]. Nevertheless, based on most clinical and experimental evidence, weight loss stabilization and nutritional support improve patient quality of life and prolong the survival of ALS animals.

The first animal model of ALS was created in the mouse, encoding a mutation found in fALS that converts glycine residue 93 to an alanine (G93A) in the SOD1 protein [24]. That model shows pathogenesis of muscle paralysis similar to that observed in clinical cases and all the histopathological hallmarks observed in fALS and sALS. Other mutant SOD1 mouse models include G37R, G85R, G127X, D90A and H46R. Mainly based on disturbances in RNA metabolism and protein homeostasis, mutations in genes encoding TAR DNA-binding protein 43 (TDP-43), FUS/TLS, TAF-15, ubiquilin 2 and C9ORF72 have also been investigated [25] and a number of animal disease models generated. Among these, transgenic mice carrying a human TDP-43 mutation (A315T) develop features similar to those of ALS, and progressive motor neuron (pmn) neuronopathy and wobbler mice are two other animal models in use. The pmn model resembles the SOD1 model, with hind-limb paralysis and progressive motor neuron degeneration due to a defect in microtubule function for axonal transport [26]. So far, preclinical drug testing has primarily been performed in a high-copy SOD1G93A mouse model of ALS. We therefore collected data from preclinical animal studies in a high-copy SOD1G93A mouse model mainly using the guidelines established by Ludolph *et al.* [27] In general, the mortality, onset and progression of disease (behavioral signs and weight loss) and/or histological changes had been performed in the drug-treated group comparing with the vehicle group. In this review, we compared and summarized the reliable results from different laboratories and research sites. Our focus was to select agents that attenuate bodyweight loss, improve muscle function and produce a significant change in onset, progression or mortality.

Mechanism-based preclinical drug development

The complex mechanisms underlying ALS pathology include inflammation, oxidant stress, apoptosis, mitochondrial dysfunction, excitotoxicity and SOD1 protein aggregation. We will compare the effects of drugs targeting these mechanisms on bodyweight loss, muscle damage, disease onset, duration and survival (Table 1, Fig. 1).

Bodyweight loss occurs in ALS experimental animals and in human patients, owing in a large part to muscle atrophy. However, bodyweight is not only a sign of ALS onset but also directly correlated to prognosis, and has often been used to evaluate therapeutic benefits. Rotarod performance is often used to determine the onset of ALS disease and assess muscle function in preclinical animal studies. In addition, the following neurobehavioral tests are commonly used: (i) postural reflex [28]; (ii) balance beam [29]; (iii) screen and paw-grip endurance (also known as the hanging wire test); (iv) tail suspension or extension reflex [30]; (v) footprint analysis [31]; and (vi) Basso-Beattie-Bresnahan locomotor rating [32].

Anti-inflammatories

Although motor neuron death is the hallmark of ALS, the concept of 'non-cell-autonomous' neurodegeneration has been applied to ALS [33], and studies indicate that non-neuronal cells including microglia, astrocytes and Schwann cells interact with damaged motor neurons and each other to mediate and affect ALS disease [34,35]. Interestingly, the loss of motor neurons in transgenic mouse models of ALS [36,37] and in ALS human postmortem tissues [38–41] are accompanied by a robust glial reaction and proliferation, as well as microglial activation. Increasing evidence shows that neuroinflammation involving microglia and astrocytes contributes to disease progression [42] and motor neuron damage in ALS [43], and when associated with astrocytes and microgliosis leads to the release of proinflammatory cytokines [44] such as interleukin (IL)-6 [45]. ALS-linked mSOD1 activates caspase-1 and IL-1β in microglia, which has been implicated in neuroinflammation [46]. In addition, there is significant elevation in tumor necrosis factor (TNF)α and Fas ligand immunoreactivity in ALS mice and human patients [47]. Because inflammation is fundamental to the pathogenesis of ALS, anti-inflammatories could play an important part in treatment.

Celastrol, derived from a traditional Chinese herb, is a triterpene originally used as an anti-inflammatory, but also has strong antioxidative effects and increases expression of heat shock protein (HSP)70 [48]. Celastrol was administered orally to G93A mice starting at 30 days of age; 2 and 8 mg/kg/day increased survival by 9.4% and 13%, respectively, and significantly reduced weight loss, improved motor function and delayed disease onset [49]. In treated animals neuronal cell count was increased by 30% in the lumbar spinal cord and TNFα, inducible nitric oxide synthase (iNOS), CD40 and glial fibrillary acidic protein (GFAP) immunoreactivity was reduced.

Cannabinoid receptor 2 (CB2) is upregulated in microglia in response to inflammatory stimuli [50], and CB2 agonists suppress microglial activation *in vitro* [51]. Cannabinoids produce anti-inflammatory effects through CB2. The CB2 cannabinoid agonist AM-1241 prolongs survival of G93A mice when delivered at symptom onset [52] and delays disease progression [53,54]. AM-1241 improves the motor performance of G93A mice (especially males) but has no protective effect on bodyweight in mice of either sex [53].

TNFα and Fas ligand immunoreactivity is greatly elevated in lumbar spinal cord motor neurons and glial cells in transgenic ALS mice and ALS patients [47]. Thalidomide works as an anti-inflammatory by destabilizing the mRNA of TNFα and other cytokines. Treatment was administered orally with 50 or 100 mg/kg/day in a transgenic G93A mouse model at 30 days of age. Thalidomide delayed onset significantly improved motor performance from 98 to 155 days of age [47], and attenuated weight loss from the age of 70 days. Survival was prolonged in a dose-dependent manner from 130 days to 145 days (12%, 50 mg/kg) or 151 days (16%, 100 mg/kg), associated with reduced TNFα and Fas ligand immunoreactivity in the spinal cord. Similarly, lenalidomide has been used to inhibit inflammatory signals caused by ALS pathology in G93A mice; it is less potent at reducing TNFα but more potent at reducing other proinflammatory signals (e.g. Fas ligand, IL-1β, TNFα and CD40 ligand) [47]. One study treated presymptomatically [47] and the other treated at symptom onset [55]. Presymptomatic oral treatment with 100 mg/kg/day started at 30 days of age significantly increased survival from 130 days to 154 days (18.5%). In the subsequent study, lenalidomide at the same dosage

TABLE 1

Summary of agents for amyotrophic lateral sclerosis (ALS) with individual neuroprotective mechanism.

Agent	Model	Delivery/dosage	Muscle function	Onset	Mortality	Refs
Anti-inflammatory agents						
Celastrol	G93A/B6SJL	Oral 2 mg/kg 8 mg/kg	Motor performance (+)	95.8 ± 11 (Veh) vs 109.5 ± 8 (2 mg/kg)	128.8 ± 5.6 (Veh) vs 140.8 ± 7.8 125 ± 7 (Veh) vs 141 ± 8	[49]
AM-1241	G93A/B6SJL	IP at onset 0.3 mg/kg 3 mg/kg	Motor performance and score (+)	IP at onset 90 days IP at onset 75 days	113.7 ± 1.7 (Veh) vs 123.4 ± 2.2 (0.3 mg/kg) vs 126.9 ± 2.8 (3.0 mg/kg) 123.6 ± 2.3 (Veh/male) vs 126.7 ± 1.2 (male) 134.4 ± 3.4 (Veh/female) vs 130.9 ± 3.0 (female)	[53,54] [53]
Thalidomide	G93A/B6SJL	Food 50, 100 mg/kg/day	Motor performance (+)	90 (Veh) Disease onset was delayed	130 ± 8.5 (Veh) vs 145 ± 19 (50 mg) vs 151 ± 19 (100 mg)	[47]
Lenalidomide	G93A/B6SJL	Food 100 mg/kg/day	Motor performance (+)	102 (Veh) vs 124 40.8 ± 6.7 (Veh) vs 59.1 ± 5.8 45%	130 ± 4 (Veh) vs 154 ± 16 127.7 ± 5.1 (Veh) vs 142.7 ± 5.4	[47,55]
NDGA	G93A/ B6SJL	Diet 2500 ppm	Motor performance and paralysis (+)	112 (Veh) vs 120	122 (Veh) vs 134	[56]
Pioglitazone	G93A/B6SJL	40 mg/kg/ day by food Diet 1200 ppm	Motor performance (+) Muscle fiber diameter in quadriceps muscle (+)	100 ± 8 (Veh) vs 110 ± 11 90 (Veh) vs 124	123 ± 7 (Veh) vs 133 ± 6 123.8 ± 6.8 (Veh) vs 139.9 ± 8.1	[57,58]
Antioxidant agents						
FeTCPP	G93A/B6SJL G93A/C57B6	IP 1 mg/kg/day	Motor performance (++)	101.2 ± 1.4 (Veh) vs 104.4 ± 2.9	128 ± 1.7 (Veh) vs 135 ± 2.4	[60]
DP109	G93A/ B6SJL	5 mg/kg	Motor performance (+)	93 ± 7 (Veh) vs 100 ± 6	133.5 ± 6 (Veh) vs 147 ± 10	[61]
DP460	G93A/ B6SJL	10 mg/kg	Motor performance (++)	93 ± 7 (Veh) vs 102 ± 9	133.5 ± 6 (Veh) vs 145 ± 10	[61]
M30	G93A/ B6SJL	Oral gavage 1 mg/kg, 4 times/week	General muscle strength (+)	107 ± 3 (Veh) vs 112 ± 4	124 ± 6 (Veh) vs 134 ± 12	[63]
Mitochondrial protective agents						
Cyclosporin A	G93A/ B6SJL	ICV 20 μg/week	Delays hind-limb weakness (+) Improves physical performance (+), hind-limbstrength and agility (+)	122.5 ± 3 (Veh) vs 135 ± 3.8	130 ± 3.4 (Veh) vs 146 ± 4.5	[64,65]
Olesoxime	G93A/B6SJL	SC 3 mg/kg 30 mg/kg per day	Grip strength (+)	Bodyweight loss delayed by 15 days and grid performance declined by 11 days	125 ± 3 (Veh) vs 138 ± 4 (3 mg/kg) vs 135 ± 3 (30 mg/kg)	[66]
Antiapoptotic agents						
Guanabenz	G93A/ B6SJL	4 mg/kg every other day	Motor performance (++)	104.5 ± 2.0 (Veh) vs 116.9 ± 2.4	132.2 ± 4.0 (Veh) vs 150.7 ± 4.7	[72]

TABLE 1 (*Continued*)

Agent	Model	Delivery/dosage	Muscle function	Onset	Mortality	Refs
NaPB	G93A/B6SJL	IP 300 mg/kg 200 mg/kg 400 mg/kg, per day	Grip strength bodyweight, rotarod, stride length, motor performance (++)	70 (Veh) vs 91	126.1 ± 2.7 (Veh) vs 142.2 ± 5.4 (300 mg/kg) 125.7 ± 3.0 (Veh) vs 136.5 ± 5.5 (200 mg/kg) vs 153.2 ± 6.4 (400 mg/kg)	[83] [73]
Antiexcitotoxic agents						
Riluzole + NaPB	G93A/B6SJL	IP 16 mg/kg 300 mg/kg	Forelimb grip strength, bodyweight (+)	84 (Veh) vs 100	126.1 ± 2.7 (Veh) vs 153.2 ± 9.1	[83]
Riluzole	G93A/B6SJL	IP 30 mg/kg/day 100 μg/ml 3 times per week	Grip strength, bodyweight (+)	11 (Veh) vs 14 weeks 95 ± 12 (Veh) vs 98 ± 11 ($P > 0.05$)	210.9 (Veh) vs 233.6 134 ± 8 (Veh) vs 148 ± 14	[81] [82]
SOD1 aggregation clearing agents						
Arimoclomol	G93A SOD1	IP 10 mg/kg	Fatigue index of EDL (+), contractile characteristics of EDL (+), maximum force of TA and EDL (+)	70 (Veh)	125 ± 1.8 (Veh) vs 153 ± 2.6	[88,89]

Data in table are collected, analyzed and calculated based on original published data. + improvement of behavior or muscle performance of ALS mouse after treatment of drug compared with control mouse; ++, obvious protective effect of drug on behavior or muscle performance of ALS mouse; N/A, no available data.

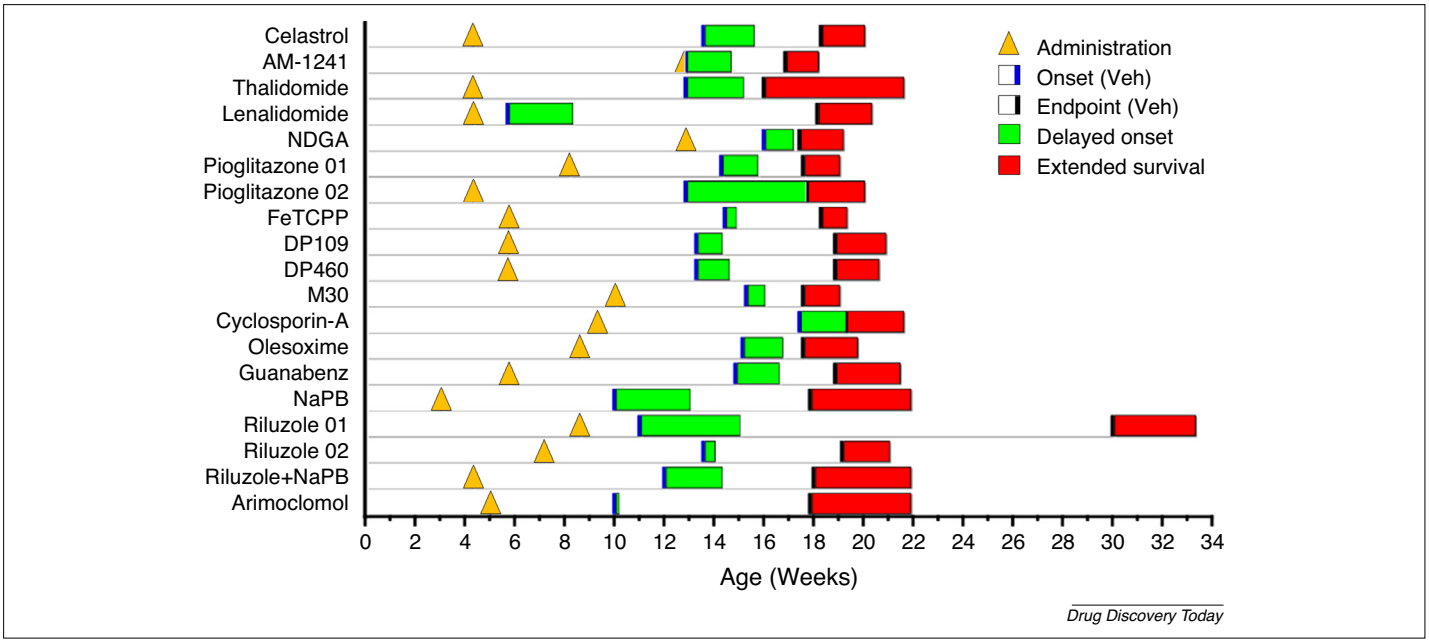


FIGURE 1

Comparison of effects of neuroprotective agents for amyotrophic lateral sclerosis (ALS) disease in preclinical study. Neuroprotective agents were selected based on their ability to attenuate bodyweight loss, improve muscle function and significantly delay onset and mortality. Selected agents are displayed on the vertical axis. Animal model lifespan is displayed on the horizontal axis in weeks, with depicted age of administration (orange triangle), onset (blue) and endpoint (black). The time frames between treated group and vehicle-treated group are depicted as delay in onset (green) and extended survival (red). Riluzole 01, pioglitazone 02 and NaPB presented good efficacy on delaying onset of ALS, but only riluzole 01 and NaPB delayed mortality; pioglitazone 02 had moderate efficacy on mortality of ALS. Thalidomide, arimoclomol and riluzole combined with NaPB had good efficacy on mortality of ALS, but thalidomide and riluzole combined with NaPB elicited only moderate effects on onset of disease; arimoclomol had poor or no effects on onset of ALS. The onset of disease in most ALS animals began at age 12–14 weeks (the average was 13 weeks in selected studies), and age of administration was 4–6 weeks in our selected studies (the average was 6 weeks). AM-1241, lenalidomide, NDGA and riluzole 01 appeared to take effect in a relatively short period of time (1–3 weeks), but those selected agents just showed moderate or poor effects on onset and mortality of ALS disease except riluzole 01.

at symptom onset increased survival by only 11.7% [55]. Both treatments improved rotarod performance and attenuated weight loss and neuronal cell death in the lumbar spinal cord [47,55].

Nordihydroguaiaretic acid (NDGA), an arachidonic acid 5-lipoxygenase (5LOX) and tyrosine kinase inhibitor, was administered orally to SOD1G93A mice [56]. Increased levels of 5LOX mRNA and protein were evident at 120 days of age. Oral treatment starting at 90 days of age with 2500 ppm of NDGA significantly increased bodyweight, delayed disease onset and extended survival. Lumbar spinal cord sections from treated animals revealed reduced levels of astrogliosis and cleaved microtubule-associated tau protein. The median duration of disease was 10 days in untreated mice and 14 days in the NDGA-treated group, where median survival was extended by 14 days or 9.8%.

Pioglitazone, an agonist of a peroxisome proliferator-activated receptor (PPAR), is a neuroprotective anti-inflammatory that delays the disease process in SOD1G93A mice [57]. Administered in food presymptomatically at 30 days of age, pioglitazone significantly decreased mortality by 13% and delayed symptom onset by 34 days [58], but did not affect progression once symptoms appeared. In a similar mouse model, treatment starting at 57 days of age with an average of 40 mg/kg consumption a day was also effective: onset was delayed by 10 days and survival was increased by 10 days. Pioglitazone treatment delayed weight loss and decreased the rapid functional decline observed in SOD1G93A control mice. Compared with untreated controls, levels of CD40, GFAP, iNOS, nuclear factor (NF)-κB and 3-nitrotyrosine were all lower in the spinal cords of treated animals. Among anti-inflammatory agents, those given after onset modestly increased survival, indicating some effectiveness. However, earlier administration produced greater delays in disease development and progression.

Antioxidant agents

Oxidative damage is a key component in the pathogenesis of motor neuron degeneration in ALS, and antioxidants increase survival [59]. However, here we limit our focus to antioxidant agents that reduce bodyweight loss. Treatment with iron porphyrin (4-carboxyphenyl porphyrin, or FeTCPP) has been shown to provide modest neuroprotection in a G93A mouse model, with an overall increase in survival of 9 days (5%) [60]. Treatment prolonged disease progression 54%, a difference of 16 to 25 days. At day 113, oxidative stress markers in all groups showed substantial reduction in the gray and white matter of the lumbar spinal cord from the FeTCPP-treated mouse which is comparable with the N1029 wild-type mouse as assessed by malondialdehyde staining or measuring total protein carbonyls. FeTCPP not only improved motor performance but also prevented loss of bodyweight.

The lipophilic metal chelators DP-109 (5 mg/kg/day) and DP-460 (10 mg/kg/day) significantly delayed onset in the G93A-transgenic ALS mouse model (7.5% and 9.5%, respectively) and extended survival (10% and 9%). Compared with untreated controls, DP109 or DP460 treatment delayed progression by 7 or 3 days, respectively. There was also a reduction (although not significant) in ALS-related weight loss in both treatment groups, which is consistent with motor performance [61]. In addition, DP-109 and DP-460 reduced the markers of oxidative damage in the lumbar spinal cord of G93A mice [61].

The iron chelator M30 exerts neuroprotective effects by upregulating a number of neuroprotective mechanisms and promotes survival signaling pathways in the brain [62]. Oral gavage of M30 (1 mg/kg) to G93A high-copy transgenic mice beginning at 70 days of age delayed disease onset by 4.6%, extended survival by 8% and attenuated bodyweight loss [63].

Mitochondrial protective agents

Many reactive oxygen species (ROS) are generated during ATP formation in mitochondria. In ALS an excess of ROS can lead to release of proapoptosis molecules, causing neurodegeneration. We next focus on two mitochondrial protective agents: cyclosporine A (CsA) and olesoxime. CsA prevents mitochondrial transition pore formation and is neuroprotective. Weekly intracerebroventricular (ICV) injections of 20 μg CsA starting 65 days before onset attenuated the decline of motor performance in G93A mice, helped maintain physical performance, preserved bodyweight and extended survival by 12% [64]. A similar study began administration at 3 months, chosen as the point of late-stage onset. CsA treatment extended survival from diagnosis point to mortality by 24.2 days, compared with an 11.8-day change with vehicle alone [65].

Olesoxime targets the outer mitochondrial membrane proteins [66,67] and affects microtubule dynamics [68]. Subcutaneous injection of olesoxime to G93A mice delays loss of bodyweight by 15 days and decline in grid performance by 11 days [66]. In another study, 600 mg/kg was added to the diet starting at 21 days of age and ending at either 60 days (presymptomatic) or 104 days (symptomatic).

Antiapoptotic agents

Apoptosis is a feature of chronic neurodegenerative diseases, particularly ALS [69,70]. Understanding the pathways affected by antiapoptotic agents is an active area for future ALS therapeutic studies. The best-studied of the antiapoptosis agents is Bcl-2, the most important protein in regulating programmed cell death [69]. Overexpression of Bcl-2 delays onset, attenuates the degeneration of spinal cord motor neurons and prolongs survival in ALS mice [71].

One recent report shows that the antihypertensive guanabenz significantly increased the expression of Bcl-2 but downregulated BAX (proapoptosis) in SOD1G93A mice. Administration of 4 mg/kg every other day beginning at 40 days of age delayed disease onset by 12 days, extended lifespan by 18 days and delayed bodyweight loss in SOD1G93A mice [72].

Sodium phenylbutyrate (NaPB) is a histone decacetylase (HDAC) inhibitor. It arrests growth, induces differentiation and reduces apoptosis [73]. NaPB has emerged as a potential therapeutic drug for a broad spectrum of neurological diseases [74,75]. Intraperitoneal (IP) administration of 400 mg/kg per day significantly extended survival by 21%, improved motor performance and neuropathological phenotype and ameliorated bodyweight loss in G93A mice. NaPB treatment also reduces the release of cytochrome c and subsequent induction of activated caspase-9 and caspase-3 activity in ALS mice [73].

Antiexcitotoxic agents

Another explanation for neurodegeneration in ALS is glutamate-mediated toxicity [76]. A disturbance in astrocytic glutamate

transport was shown to elevate levels of glutamate in the cerebrospinal fluid of ALS patients [77], and glutamate-mediated toxicity is noted in ALS [76]. G85R, A4V and I113T SOD1-mutant mouse lines show a marked loss or inactivation of glutamate transporters [78,79]. Evidence from clinical and animal models supports excitotoxic death of motor neurons as a mechanism in ALS pathogenesis.

Riluzole, the only FDA-approved ALS treatment, prolongs patient life by only a few months [80], with the proposed mechanism being antiexcitotoxicity. Riluzole delays onset of muscle weakness and extends lifespan by 4–6 weeks in animal models of ALS [81,82]. When 16 mg/kg of riluzole was combined with 300 mg/kg of NaPB, survival was increased over either treatment alone: NaPB increased survival by 12.8%; riluzole 7.5%; the combination extended survival 21.5%. All groups had increased bodyweight and grip strength compared with untreated animals, with the combination group showing the greatest increase [83].

Antiaggregation agents

Misfolded proteins and abnormal aggregation of proteins are pathologically characteristic of neurodegenerative diseases including ALS [84,85]. Misfolded proteins are often thermodynamically stable and form aggresome-like structures; ALS progression can correlate with a propensity toward protein aggregation. The heat shock response (HSR) is a highly conserved cytoprotective mechanism; enhanced HSP expression not only directly affects protein

TABLE 2

Neuroprotective agents delay bodyweight loss in animal models of amyotrophic lateral sclerosis (ALS).

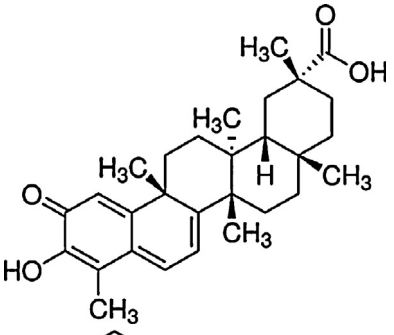
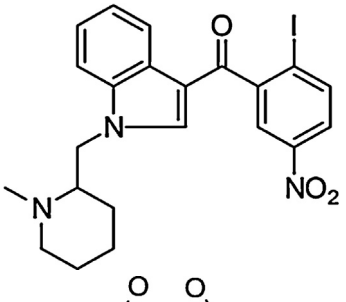
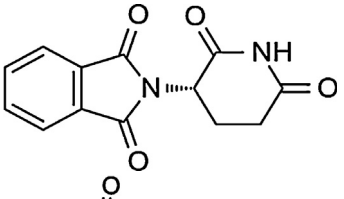
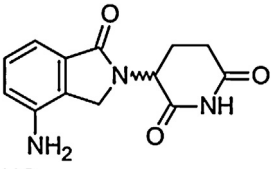
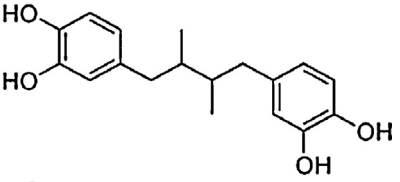
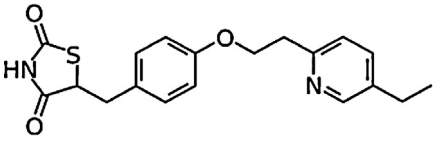
Mechanism	Bodyweight	Chemical structure of agent	Refs
Anti-inflammatory agents	(+)		[49]
	(+)		[53]
	(+)		[47]
	(+)		[47,55]
	(+)		[56]
	(++)		[57,58]

TABLE 2 (*Continued*)

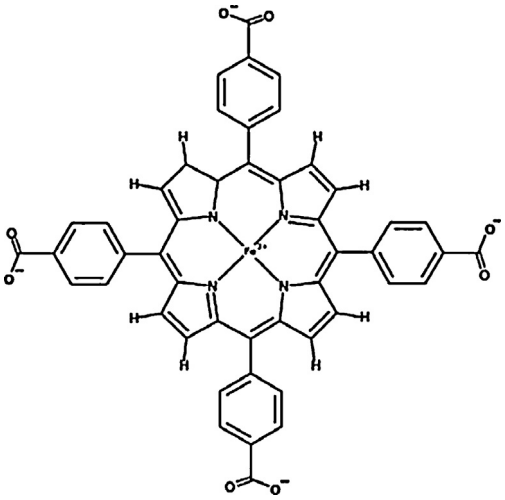
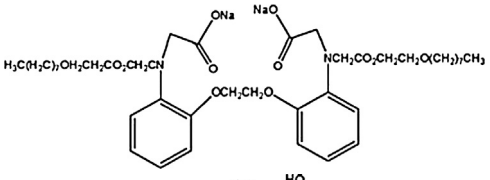
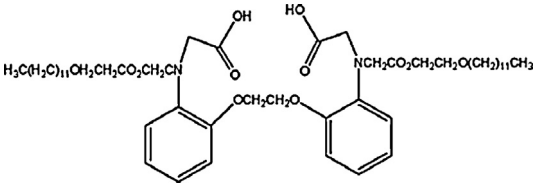
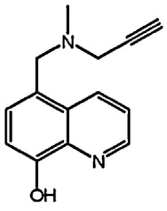
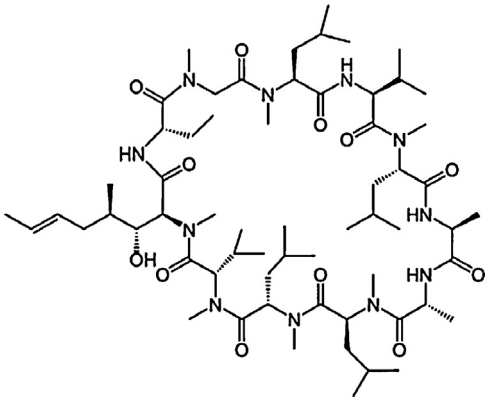
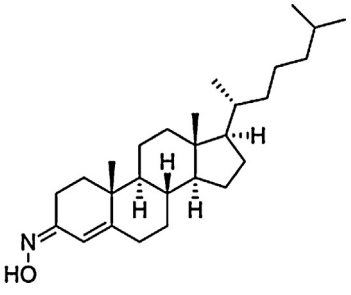
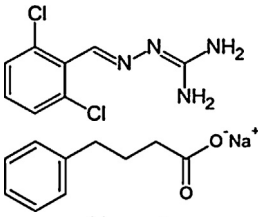
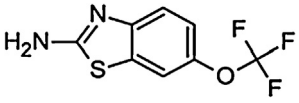
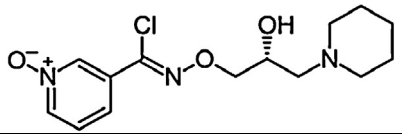
Mechanism	Bodyweight	Chemical structure of agent	Refs
Antioxidant agents	(++)		[60]
	(+)		[61]
	(+)		[61]
	(+)		[63]
Mitochondrial protective agents	(+)		[64]
	(+)		[66]

TABLE 2 (*Continued*)

Mechanism	Bodyweight	Chemical structure of agent	Refs
Antiapoptotic agents	(+)		[72] [73]
Antiexcitotoxic agents	(++)		[81–83]
SOD1 aggregation clearing agents	(+)		[88]

Neuroprotective agents inhibiting bodyweight loss and their major action mechanisms. + drug with modest increase of body weight of ALS mouse; ++, robust effect of drug on body weight gain.

aggregation but also leads to more-effective clearance of protein aggregates via the unfolded protein response [86,87].

The HSR is modulated by stress-inducible heat shock transcription factor-1 (Hsf-1). Arimoclomol, a co-inducer of HSPs, significantly delays disease progression in SOD1G93A mice [88,89]. Mice were treated with IP 10 mg/kg of arimoclomol daily at 35 days (before onset) or at 70 days of age (onset), extending survival by 22% or 18%, respectively. Progression was measured using electrophysiological assessment of hind-limb muscle function. At 120 days of age the untreated mouse measured 8.3 motor units compared with the wild-type mouse at 28 units. Treatment elicited a significant increase (14.3 units) in motor unit survival [88]. There was a 74% increase in motor neuron cell count in the sciatic motor pool of lumbar spinal cord sections of treated animals at 120 days of age. Neuroprotection was probably associated with the prolonged activation of Hsf-1, and in spinal cord sections HSP70 and HSP90 levels were increased.

Structure-guided drug screening

In ALS patients, greater loss of bodyweight has been associated with poorer prognosis [18]. Because early changes in bodyweight are significantly correlated with progression of symptoms and survival [90], we discuss agents that prevent bodyweight loss in animal models (Table 2). Very interestingly, the six anti-inflammatory agents reviewed were all found to reduce bodyweight loss and improve motor performance and survival in ALS mice. We further analyze the structure–activity relationship of the small molecules in Table 2. Despite differences in their action mechanisms and original design, some of their chemical structures display common characteristics.

Among the six anti-inflammatory agents that increased bodyweight, the chemical structures (ranging from core skeletons to substitution functionalities) of NDGA, AM-1241, pioglitazone, thalidomide and lenalidomide vary. Five of the anti-inflammatory agents have obvious common features (Fig. 2): (i) potential chelating properties with cations to form 5- to 6-membered rings that are thermodynamically stable; (ii) antioxidation moiety, phenol or different amines; and (iii) strongly negatively charged atoms as hydrogen-bond acceptors.

These characteristics are common not only to the anti-inflammatory agents but also to the other small molecules in Table 2. For instance, antioxidants and anti-inflammatories share some chemical structures. DP109, DP460 and M30 are classified as antioxidants; DP109 and DP460 are analogs with the same core skeleton and different-length side-chains; both exhibited structural features distinct from M30, whereas all three possess: (i) chelating properties with cations to form 5- or 6-membered rings that are thermodynamically stable (Fig. 2); (ii) antioxidation properties at

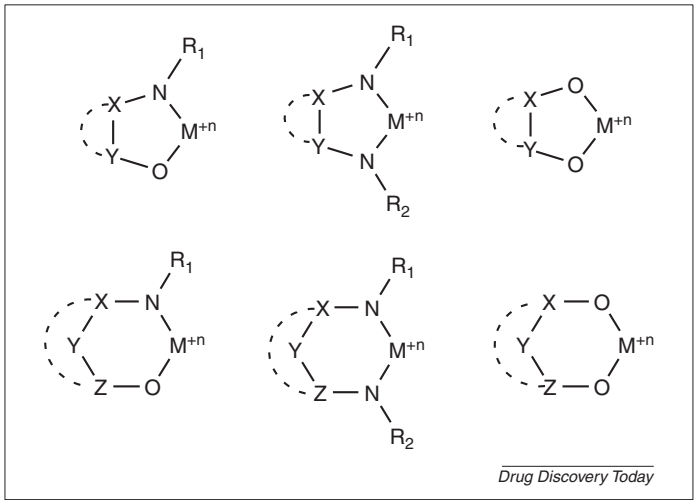


FIGURE 2

Thermodynamic stable chelating complexes formation of positive cationic with amyotrophic lateral sclerosis (ALS) potential heteroatom therapeutics. Three types of 5-membered ring metallic complexes are shown in the upper panel through the lone pairs or negatively charged oxygen or nitrogen atoms; oxygen represents hydroxy, ether, carboxylate, etc., and nitrogen stands for primary amine, secondary amine, tertiary amine, imine, enamine, etc., which are all strong electron-donating functionalities. Three other types of 6-membered ring metallic complexes are shown in the lower panel, where oxygen and nitrogen have the same or similar functionalities as in the upper panel. The functionalities attached to therapeutic reagents readily chelate metallic cations. M^{n+}: Cu^{2+}, Fe^{2+}, Cu^{1+} and all excess redox-active transition metallic cations. X/Y/Z: aliphatic C, aromatic C or heteroatom (e.g. S, P, N, etc.). R1/R2: H, aliphatic carbon side-chain and aromatic carbon. Broken line: aliphatic side-chain, aliphatic cyclic fused ring and aromatic fused ring.

phenol and aniline moieties; and (iii) hydrogen binding to protein or enzyme receptors. Thus, combining chemical structural analysis with the knowledge that certain small molecule compounds can increase bodyweight and improve motor performance and survival could be a feasible strategy with which to search for drug candidates for ALS treatment based on ameliorating bodyweight loss.

Concluding remarks and future perspectives

Riluzole has been demonstrated to have a beneficial effect in people with ALS as well as in SOD1G93A mice. At the same time, its effects are modest and other scientifically promising therapeutic candidates exist. Unfortunately, many previously promising candidate therapeutics have shown beneficial effects in the SOD1G93A mouse model of ALS only to meet with a lack of benefit when tested in people with ALS. Examples include thalidomide [91] and olesoxime, which was well tolerated but failed to show efficacy in a large Phase II–III clinical trial [92], and pioglitazone, which did not show survival benefit in a Phase II trial [93]. These examples highlight the poor track-record of translation from survival studies in the SOD1G93A mouse model into humans.

However, even as evidence mounts that survival studies in the mouse model might have a poor positive predictive value for human clinical trials, *in vivo* leads have also fallen short for predicting success in human trials, and the SOD1G93A mouse model has become even more entrenched as a preclinical screening tool, guiding the choice of promising therapeutic agents to carry forward to human trials.

Our review summarizes changes in bodyweight and muscle function, demonstrates that these outcome measures in mouse studies have been underutilized and suggests that bodyweight and muscle function should be relied upon more heavily in future studies in the ALS mouse model. Rather than relying heavily on survival, more-comprehensive criteria for the selection of promising ALS therapy candidates might include: (i) significant prolongation of survival and delay in onset of weakness; (ii) survival prolongation after disease onset because most people with ALS are diagnosed after onset the window of prevention has closed and the goal of therapy must be to slow disease progression, rather than staving off symptom onset; (iii) delay in bodyweight loss; and (iv) improvement of muscle functions.

Because ALS is a multifaceted pathological disease, various pleiotropic agents and exciting new therapies targeting one or more molecular pathogenic mechanisms are currently being studied [94,95]. Novel therapeutic agents could even have differential effects at different times in the progression of disease. Furthermore, there could be numerous pathologically and/or genetically distinct forms of the disease, which might be best targeted with distinct therapies. In addition, some agents shown to improve muscle function failed extended survival in ALS animals [96–99].

As a result, a broad range of selective therapeutics might each have a minimal effect on the whole population of ALS, or a substantial effect in one patient subgroup and virtually no effect in another subgroup. This could suggest that, with appropriate animal models, treatment with combinations of therapies could be better explored. Motor-neuron-targeted agents might be combined with other classes of drugs, for example skeletal-muscle-targeted treatments, to increase effectiveness.

Given the complexity of the disease, the likelihood of a complex solution emerging is high. Solving such a complicated problem will require collaboration between academia and biotech and pharmaceutical industries. Furthermore, biomarker discovery might help us to recognize the earliest symptoms of ALS, ultimately improving prognosis and contributing to clinical trials with ALS patients. Ongoing therapeutic trials in animal models of ALS will provide insight into promising therapies to decrease bodyweight loss, improve motor performance, extend survival and delay disease progression. Our review could imply a novel, practical and feasible strategy for drug discovery applicable to ALS.

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