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Potential new complication in drug therapy development for amyotrophic lateral sclerosis

Svitlana Garbuzova-Davis, Ph.D., D.Sc.[1,2,3,4,*] **[Associate Professor]**, **Avery Thomson, M.S.**[5] **[Clinical Research Specialist]**, **Crupa Kurien, B.S.**[1] **[Research Assistant]**, **R. Douglas Shytle, Ph.D.**[1,2] **[Associate Professor]**, and **Paul R. Sanberg, Ph.D., D.Sc.**[1,2,4,6] **[Distinguished Professor, Executive Director of CABR]**

[1]Center of Excellence for Aging & Brain Repair, University of South Florida, Morsani College of Medicine, Tampa, Florida 33612, United States of America

[2]Department of Neurosurgery and Brain Repair, University of South Florida, Morsani College of Medicine, Tampa, Florida 33612, United States of America

[3]Department of Molecular Pharmacology and Physiology, University of South Florida, Morsani College of Medicine, Tampa, Florida 33612, United States of America

[4]Department of Pathology and Cell Biology, University of South Florida, Morsani College of Medicine, Tampa, Florida 33612, United States of America

[5]Department of Neurology, University of South Florida, Morsani College of Medicine, Tampa, Florida 33612, United States of America

[6]Department of Psychiatry, University of South Florida, Morsani College of Medicine, Tampa, Florida 33612, United States of America

Abstract

Introduction—Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by motor neuron degeneration in the brain and spinal cord. Treatment development for ALS is complicated by complex underlying disease factors.

Areas covered—Numerous tested drug compounds have shown no benefits in ALS patients, although effective in animal models. Discrepant results of pre-clinical animal studies and clinical trials for ALS have primarily been attributed to limitations of ALS animal models for drug-screening studies and methodological inconsistencies in human trials. Current status of pre-clinical and clinical trials in ALS is summarized. Specific blood-CNS barrier damage in ALS patients, as a novel potential reason for the clinical failures in drug therapies, is discussed.

[*]Author for correspondence: Svitlana Garbuzova-Davis, Ph.D., D.Sc., Center of Excellence for Aging and Brain Repair, Department of Neurosurgery and Brain Repair, University of South Florida, Morsani College of Medicine, 12901 Bruce B. Downs Blvd., Tampa, FL 33612, Tel: 813-974-3189, Fax: 813-974-3078, sgarbuzo@health.usf.edu.

Declaration of interests

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Expert opinion—Pathological perivascular collagen IV accumulation, one unique characteristic of barrier damage in ALS patients, could be hindering transport of therapeutics to the CNS. Restoration of B-CNS-B integrity would foster delivery of therapeutics to the CNS.

Keywords

ALS; animal models; blood-CNS barrier; clinical trials; drug therapy; patients

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by gradual motor neuron degeneration throughout the neural axis. The rapid progression of debilitating symptoms of ALS leads to paralysis and fatality [1,2]. Approximately 50% of patients die within 30 months of disease symptom onset and only 20% of patients survive 5 to10 years after symptom onset [3]. In the United States, estimated ALS incidence and prevalence are 5.4/100,000 and 10.5/100,000, respectively [4,5] with an estimated 5,000 new cases annually [6]. ALS cases are 90–95% sporadic (SALS) with the remaining 5–10% of cases being genetically linked, familial (FALS). Peak ages for disease onset are 58–63 years for SALS and 47–52 years for FALS [7]. Men have a higher incidence of this disease than women. The overall population-based lifetime risk for ALS is 1:350 for men and 1:400 for women. Within FALS cases, approximately 20% result from missense mutations in the genes coding for Cu/Zn superoxide dismutase (SOD1) [8,9] and about 2–5% have mutations of the TARDBP gene encoding the TAR-DNA binding protein TDP-43 [10]. Additional gene mutations in FUS [11,12] and ANG [13], approximately 5% and 1% of FALS cases respectively, have been identified. Relatively recently, a mutation in the C9ORF72 gene was identified and has been associated with a significant number of FALS, FALS with frontotemporal dementia, and SALS cases [14,15]. The clinical presentation and underlying pathology of SALS and FALS are similar and treatment options for ALS patients are limited and mainly supportive. The only FDA approved drug to treat ALS is riluzole (Rilutek®), which blocks glutamate release and extends the lifespan of ALS patients by only a few months [16].

Since French neurologist Jean-Martin Charcot first described ALS as a rapidly progressive neuromuscular disease in 1869 [17], there have been growing clinical and scientific efforts to understand the disease pathogenesis. Numerous hypotheses about the etiopathology of ALS have been proposed (reviewed in [18–29]), including glutamate excitotoxicity, mitochondria dysfunction, oxidative stress, glial cell pathology, impaired axonal transport, protein aggregations, immune reactivity, neurotrophic factor deficits, and neuroinflammation.

Development of an effective treatment for ALS is complicated by the diffuse nature of motor neuron degeneration and by the complexity of intrinsic and extrinsic factors underlying this disease. Although more than 30 drug compounds have been tested in ALS clinical trials, most drugs failed to show benefit in ALS patients [30,31].

The present review briefly summarizes current status and potential pitfalls of pre-clinical and clinical trials in ALS. A new potential cause for the observed ALS clinical trial failures is discussed.

2. Power of pre-clinical studies in successful clinical trials for ALS

The therapeutic strategies for ALS have primarily focused on pharmacological interventions to prevent and/or delay motor neuron deterioration and thereby slow or arrest disease progression. These disease-modifying neuroprotective strategies target motor neurons. However, none of the agents tested for their neuroprotective role in human studies demonstrated a significant benefit in survival or quality of life of ALS patients, although each of these compounds showed promising results in animal models. For example, brain-derived neurotrophic factor (BDNF) and ciliary neurotrophic factor (CNTF) as potent drugs for ALS failed to demonstrate efficacy in clinical trials [32–34], although improvements in motor neuron protection were demonstrated in preliminary studies of axotomized rats or wobbler mice as relevant models of ALS [35–38].

Similarly, insulin-like growth factor-1 (IGF-1), including the recombinant form, delivered intrathecally or intraparenchymally into a superoxide dismutase 1 (SOD-1) rodent model of ALS demonstrated beneficial effects by prolonging survival and increasing motor function in a number of reports [39–42]. However, the reported improvement in SOD-1 animal survival was controversial [43,44]. Despite the controversy, intrathecal [45] and subcutaneous [46] administration of IGF-1 were investigated in later clinical trials. Although some benefits were shown in patients receiving a high dose of IGF-1 via intrathecal delivery, ALS patients with subcutaneous drug treatment showed no improvement in Phase III clinical trials. It has been suggested that intrathecal IGF-1 treatment might have clinical significance but development of better drug delivery mechanisms strength “important for validating the utility of IGF-1 as a therapy for ALS” [47]. Detailed discussions regarding pre-clinical and clinical trials of the above mentioned and other growth factors (glial-derived neurotrophic factor (GDNF), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), etc.) are available [48,49]. It is believed that neurotrophic growth factors, which might promote motor neuron survival, are the most rational therapeutic approach for ALS.

Minocycline, a tetracycline antibiotic, also demonstrated positive effects in animal studies by delaying disease progression and extending lifespan in SOD-1 mutant mice [50–52]. This beneficial drug effect resulted from attenuated microglial activation in the spinal cord of treated animals at the end stage of disease. However, results of a Phase III clinical trial were negative and escalated doses of minocycline even proved harmful [53,54]. A reasonable question was raised by Keller at al. [55] regarding glial cells as a potential therapeutic target in ALS. The authors performed a study to evaluate the effect of minocycline treatments initiated at different stages of disease in SOD-1 G93A mice. Results of this study, showing altered astrocyte reactivity and increased microgliosis at late stage of disease, suggest that minocycline lacks anti-inflammatory actions.

Similarly to minocycline treatment, creatine monohydrate, a potential cellular energy supplier, showed no obvious therapeutic benefit on survival or disease progression in ALS

patients in double-blind placebo-controlled studies [56–58], although benefitting motor function and survival of transgenic mice modeling ALS [59].

Additionally, celecoxib, a cyclooxygenase-2 inhibitor, which reduced astrocytic glutamate release, was effective in increasing motor neuron survival and prolonging lifespan of G93A mice [60], but showed no benefits in a double-blind, placebo-controlled clinical trial [61]. Another compound, ceftriaxone, efficiently prolonged survival of G93A animals via increased glial glutamate transporter expression in astrocytes [62]. Nevertheless this drug did not show clinical efficacy in a Phase III clinical trial [63]. Significantly more adverse gastrointestinal and hepatobiliary events were detected in ALS patients treated with ceftriaxone than in the placebo group.

Also, memantine, a noncompetitive NMDA receptor antagonist and a neuroprotective agent against glutamate-induced toxicity, initially demonstrated therapeutic efficacy in G93A SOD 1 mice by significantly delaying disease progression and increasing lifespan of mice [64], even when administered into symptomatic animals [65]. When this drug was evaluated in a Phase II/III double-blind, randomized clinical trial for 12 months, there were no adverse events but efficacy of memantine was not observed [66]. Interestingly, another multi-center double-blind, placebo controlled pilot study investigating the effect of memantine in ALS patients who took riluzole by examining changes in CSF biomarkers demonstrated that the drug was well tolerated with declines in CSF tau levels, even to healthy control levels in some patients [67]. Thus, combined treatment of memantine with riluzole might be efficacious for ALS patients. However, the effect of this combinatory treatment in an animal model of disease was not determined.

Since riluzole, an antiglutamaterdic agent, is the only FDA approved drug to treat ALS patients [16], combining riluzole with new compound(s) might be an effective therapeutic strategy. Early studies by Gurney et al. [68,69] showed that riluzole significantly maintained motor function and prolonged survival in a transgenic mouse model of ALS, but disease onset was not delayed. The effect of riluzole on motor function in mice was better in early-stage disease than later [69], suggesting that early drug intervention in ALS patients might be beneficial [70]. Supporting this suggestion, reduced effectiveness of riluzole in enhancing glutamate uptake was noted at end-stage of disease in a transgenic rat model of ALS [71]. Although riluzole modestly prolongs survival of ALS patients by 4–6 months [72,73], there is an urgent need to develop therapy benefiting both survival and quality of life for ALS patients,

Recently, a randomized, placebo-controlled, double-blind Phase IIb trial evaluated the safety and efficacy of tirasemtiv, a fast skeletal troponin activator, for 12 weeks in ALS patients from 73 centers in eight countries [74]. The application for this clinical trial was based on beneficial results demonstrating muscle and motor function improvement in the G93A SOD1 mouse model [75]. However, no drug effect was shown in the changes in ALSFRS-R from baseline but losses of vital capacity and muscle strength were significantly slowed in tirasemtiv vs. placebo group. Yet, serious adverse events were determined more frequently in the tirasemtiv group. Besides the negative study results, the authors suggest “a potentially

important effect of tirasemtiv warranting further evaluation over a longer period in ALS" [74].

The above descriptions of drug therapies in animal studies and ALS clinical trials are illustrative, rather than comprehensive. However, the obvious discrepancy between results of pre-clinical animal studies and following clinical trials is disappointing. Is the dismal record of ALS clinical trials due to inadequacies in the animal models or are weaknesses endemic in the designs of these clinical trials?

Transgenic animal models of ALS carrying SOD-1gene mutations are widely used in translational treatment research. Although ALS pathogenesis in these animal models seems to mimic human disease, the rapid course of disease progression is only one of several challenges to accurate evaluation of experimental drug efficacy. To improve pre-clinical study research, guidelines for standardized drug testing methods in rodent models of ALS have been provided [76]. Importantly, consistent methodologies, including those for toxicity and dose-response effects, should be applied in pre-clinical investigations. In agreement with Scott et al. [77], confounding biological variables should be also considered in designing and interpreting drug efficacy studies, particularly investigations using G93A SOD-1 mice, which carry approximately 23 copies of the human SOD-1 G93A transgene. Interestingly, after identifying the most critical biological variables and retesting the efficacy of various compounds in G93A mice using an optimized study design, the authors found no survival benefit in mice for minocycline, creatine or other tested drugs. They stated that the majority of studies showing previous beneficial effects were "most likely measurements of noise in the distribution of survival means as opposed to actual drug effect" due to the presence of uncontrolled confounding variables [77]. The authors provided recommendations on improved pre-clinical survival study design for therapeutic drug testing in the G93A mouse. Also, pharmacodynamic and pharmacokinetic data on drugs tested in animal disease models might help identify useful agents for future ALS clinical trials [31]. Additionally, the majority of ALS studies using animal models began treatment prior to symptom onset, so investigations treating animals at late symptomatic disease stage should have increased translational value [48,78,79]. However, some reviews [79–81] have noted that the current FALS animal models may lack utility for identifying therapeutic agents and/or evaluating drug efficacy for clinical trials of SALS patients.

Numerous comprehensive reviews have deliberated the limitations and future directions of pre-clinical studies and clinical trials for ALS [29–31,48,49,78,79,82–84]. In remarks on ALS clinical drug trials, the failures were largely attributed to methodological pitfalls including: insufficient numbers of enrolled participants, heterogeneous patient populations, short duration of treatment, discrepant timing of patient drug treatments accordingly to their disease stages (vs. pre-clinical studies), absence of placebo-controlled patient groups, lack of clinically implemented specific biomarkers in evaluation of drug efficacy, and others. However, there is also ongoing debate regarding the optimal cellular target for ALS therapy. Ludolph and Jesse [82] noted that rational pharmacological intervention in disease-modifying strategies aims to prevent further deterioration of motor neurons throughout the neural axis. In this view, a current Phase II randomized, double-blind pharmacodynamic trial (NCT02450552) is evaluating the effect of oral ezogabine (retigabine) treatment on upper

and lower motor neuron excitability in ALS patients. The effect of two doses of retigabine, a potassium channel opener, vs. placebo is determined by transcranial magnetic stimulation and threshold tracking nerve conduction studies. This clinical trial could be beneficial since pre-trial *in vitro* study results showed blocking of hyperexcitability and improving motor neuron survival using induced pluripotent stem cell-derived motor neurons from ALS patients harboring mutant SOD1 [85].

However, as the disease progresses, motor neurons become subject to neuroinflammation via reactive astrocytes and activated microglia. Hence, glial cells might be a potential therapeutic target and initiation of this targeted drug treatment could depend on disease stage. Hereafter, one consideration for drug intervention studies should be selection of the appropriate cellular target due to evolving dynamic interactions between motor neurons and glial cells [78]. Also, as Kiernan et al. [1] note: “There is a crucial need to formulate therapies that not only slow disease progression, but also deal with the secondary consequences of malnutrition and respiratory failure.” Therefore, the development of effective drug therapies for ALS has a major challenge due to multiple and variable targets for therapeutic interventions. A combined drug treatment, “cocktail therapy”, is a likely and promising next step for clinical trials in ALS [29,79,83]. Even combinations of approved drugs using in treatment of other diseases might serve as potential therapeutic agents for ALS [86]. Also, gene therapy might have a significant role in treatment development for ALS by delivering various therapeutic molecules using viral/non-viral vectors across the blood-brain and blood-spinal cord barriers.

Although major problems have been identified in both pre-clinical research and clinical trials in ALS, *solid* animal drug-screening studies are necessary to select drugs for future human studies. Since 2010, a zebrafish genetic model of ALS has been widely used for drug testing [87,88]. This simple animal model for ALS might facilitate screening of chemical agents and even drug discovery via various genetic manipulations revealing new therapeutic targets [89]. Although the zebrafish model of ALS has numerous advantages for pre-clinical drug screening, some neuroanatomical differences were noted in the motor system vs. humans [90]. Also, it has been shown that zebrafish display a mature blood-brain barrier (BBB) in cerebral microvessels between 3 and 10 days post-fertilization and both structural and functional characteristics of BBB are similar to that of mammals [91–93]. However, whether the BBB in zebrafish accurately reflects BBB status in humans and mimics specific BBB pathological conditions related to ALS, as discussed below, needs future investigation. Thus, improved translational investigations as well as clinical trials with proper methodological design are hallmarks for successful drug treatment in ALS. However, one aspect of any effective drug therapy for ALS needs further discussion. Since therapeutic compounds must reach their target within the CNS, they need the ability to cross the blood-CNS-barrier. However, some potent agents considered for ALS therapy such as GDNF do not cross the barrier [94] while others such as VEGF can cross this barrier [95,96]. This review considers the blood-CNS-barrier as an integrated system allowing delivery of therapeutics to the CNS rather than focuses on particular barrier transport mechanisms.

3. Is blood-CNS barrier integrity critical for drug delivery to the CNS?

The CNS is protected from entry of hazardous serum proteins by the blood-brain barrier (BBB), blood-spinal cord barrier (BSCB), and blood-cerebrospinal fluid barrier (BCSFB). These CNS barriers control cerebral/spinal cord homeostasis by selective transport of various substances [97–101]. This control is possible due to the unique structure of the microvasculature – capillaries formed by endothelial cells (BBB and BSCB) and epithelial cells of the choroids plexus (BCSFB). Brain and spinal cord capillary endothelial cells (ECs) are connected via adherens and tight junctions [102–105]. The basement membrane (i.e. basal lamina) surrounding the ECs and pericytes supports the abluminal surface of the endothelium [106,107]. Astrocyte perivascular end-feet, ensheathing approximately 95% of the vessel wall, appear to have an important role for maintenance of the BBB/BSCB [108–110]. Integrity of all BBB/BSCB elements is critical for protection of the CNS. Impairment of this cellular machinery may cause blood-CNS-barrier (B-CNS-B) breakdown.

In ALS, one possible pathogenic mechanism is impairment of the B-CNS-B. Although some early reports showed altered BCSFB permeability in ALS [111,112], later studies were inconclusive [113,114]. Relatively recently, however, compelling evidence demonstrated structural and functional alterations in the BBB and BSCB in both ALS patients and animal models [115–122]. Major findings were degeneration of ECs and astrocyte end-feet processes surrounding microvessels, extra- and intracellular edema, impairment of endothelial transport system, and dysfunction of tight junction proteins compromising BBB/BSCB integrity, resulting in vascular leakage, microhemorrhages, and hemosiderin deposits in CNS parenchyma. This vascular pathology, demonstrating impairment of all neurovascular unit components in the brain and spinal cord, identifies ALS as a neurovascular disease [123]. It is possible that the initiating pathological trigger for ALS is a dysfunctional B-CNS-B, allowing detrimental factors from the systemic circulation to penetrate the CNS and rendering motor neurons susceptible to degeneration [124]. Supporting this suggestion, BSCB breakdown has been demonstrated in pre-symptomatic SOD1 mutant mice prior to motor neuron degeneration and inflammatory changes in the spinal cord [97]. Although B-CNS-B damage might not be a causative event in ALS, it is still a significant, and possibly critical, pathologic effector.

However, the majority of findings on ALS microvascular pathology have been determined in mutant SOD-1 rodent models, which might similarly occur in FALS patients carrying the SOD-1 mutations. To determine B-CNS-B competence without involvement of mutant SOD-1, our group recently analyzed post-mortem gray and white matter microvessels of medulla and spinal cord tissue from SALS patients and showed pervasive barrier damage in these CNS areas [117]. Furthermore, similarities and differences in barrier pathology between SALS patients and an FALS animal model were noted. Numerous signs of barrier impairment (EC degeneration, capillary leakage, perivascular edema, downregulation of tight junction proteins, and microhemorrhages) are common in both mutant SOD1 animal models of ALS and SALS patients, although other pathogenic barrier alterations such as pericyte degeneration and perivascular collagen IV expansion are still unique to SALS patients [125]. Our thoughts are that compromised BBB/BSCB integrity in animal models of ALS might be due to toxic effects of mutant SOD-1 upon ECs, but the cause(s) of barrier

damage in SALS patients are still unclear. Pericyte degeneration, one noted difference between animal models and patients, might lead to the reduced capillary blood flow shown in brains of ALS patients and correlating with disease severity [126]. For instance, extensive vascular basement membrane collagen IV accumulation, 2–2.5 times higher than controls, and even collagen fiber calcifications were demonstrated in the majority of brain and spinal cord vessels from SALS patients [117]. It is possible that buildup of perivascular collagen IV occurs as a compensatory mechanism for maintenance of vascular integrity or/and due to defective regulation of the matrix metalloproteinase (MMP) pathway by damaged endothelial cells leading to a possible imbalance between MMPs and tissue inhibitors of metalloproteinases (TIMPs) [127]. It seems highly likely that abnormal perivascular collagen accumulation in SALS patients takes place over a long period of time and could present a significant obstacle to effective drug influx to the CNS. Also, thickening of the basement membrane by collagen IV accumulation would slow diffusion of various substances across the barrier [128]. Additionally, given the high oxygen consumption of motor neurons, the higher frequency of hypertension in ALS patients may reflect a compensatory increase in blood pressure in response to a thickened vascular basement membrane [129]. A population based study found that individuals whom had taken a high dose of angiotensin-converting enzyme inhibitor (a type of blood pressure medication) for at least four years had a 57% lower risk of developing ALS than the general population [130]. Thus, there is an obvious link between thickness of the vascular basement membrane and diffusion rates of various substances across the B-CNS-B.

Additionally, similarly to ALS, Alzheimer's clinical drug trials have shown a disappointingly high rate of failures – 99.6% [131]. Identified trial weaknesses include a poor understanding of mechanisms underlying most late onset dementias and difficulties recruiting and retaining study members [132]. Interestingly, biopsied brain tissue from Alzheimer's patients revealed collagen accumulation in cerebral vascular basement membranes in addition to focal necrotic changes in endothelial cells [133,134]. Recent animal studies employing the triple transgenic Alzheimer's mouse model (3xTg-AD) reported reduced cerebrovascular volume [135], increased thickening of the epithelial basal membrane, and greater collagen-IV deposition around capillaries in choroid plexus [136]. Moreover, Mehta et al. [137] noted a significant reduction in the brain uptake of [^{3}H] diazepam and [^{3}H] propranolol, and the anti-AD drug, memantine, in aged 3×TG-AD mice versus wild type mice of the same age (up to 57% in the cortex and up to 62% in the hippocampus). A slower rate of drug diffusion in this animal model is consistent with the thickening of the perivascular basement membrane confirmed by collagen IV immunohistochemistry in cortical slices from these aged transgenic mice suggesting that the reduced uptake of the drugs was likely due to impaired perivascular basement membrane. Perhaps perivascular collagen IV buildup is hindering delivery of therapeutics to the brain in both ALS and AD.

Henriques et al. [48] noted that "we have to be certain that the protein in question passes the BBB, and reaches motoneurons in sufficient concentration, and over a reasonable time period. The delivery route and formulation need to be fitted to that purpose." Although B-CNS-B related pathologies in SALS patients have been discussed in detail [125], it is important to emphasize that differences, especially perivascular collagen IV expansion, in B-

CNS-B damage between ALS patients and animal models may explain ineffective drug therapy in clinical trials for ALS. Also, pericyte degeneration, leading to capillary blood flow changes, might hinder drug delivery for treatment of ALS. Although pericyte degeneration may be difficult to combat, collagen IV accumulation can be targeted, perhaps by administration of fenofibric acid [138], ramipril [139], anti-collagen IV antibodies or alpha, alpha'9-dipyridyl [140]. Reduction in collagen IV may aid new therapeutic strategies for ALS. Thus, drug deliveries to the CNS might be impeded, or even thwarted, by pervasive B-CNS-B damage in ALS, and this barrier should be considered the initial primary therapeutic target in any treatment approach for this disease.

4. Conclusion

ALS is a complex multifactorial disease and the development of effective drug therapies is challenged by multiple therapeutic targets. Disease-modifying strategies for ALS primarily focus on pharmacological interventions to prevent and/or delay further motor neuron deterioration and thereby, slow and/or arrest disease progression. However, none of the potent drugs in human studies significantly prolonged survival or improved patient quality of life, although these agents showed beneficial results in ALS animal models. This discrepancy between pre-clinical and clinical trials in drug efficacy for ALS is disappointing. Numerous reports have addressed the limitations in both animal studies and clinical trials for ALS. Mainly, the utility of current animal models of ALS for drug-screening studies has been questioned as these animals have imperfectly represented human disease outcomes. The primary shortcomings of ALS clinical trials have been identified as methodological inadequacies and/or inconsistencies between the various trials. The disconnect between effective drug therapies in ALS mice and unsuccessful drug treatments in ALS patients might be also point to more complex mechanisms underlying sporadic disease. Supporting this possibility, differences in blood-CNS barrier alterations have been shown between sporadic ALS patients and animal models of disease. Significant perivascular collagen IV accumulation, determined only in ALS patients, seems likely to restrict drug influx to the CNS, thereby attenuating therapeutic strategies in ALS clinical trials. Similarly to ALS, collagen IV accumulation may impact AD therapies. Thus, the blood-CNS barrier should be considered as a primary therapeutic target prior to development of any treatment approach for ALS as well as AD.

5. Expert Commentary

The mismatch between results of pre-clinical studies and ALS drug clinical trials is challenging for development of effective treatments. Although weaknesses have been identified in both animal studies and clinical trials, the key weakness is the failure to translate research findings into viable clinical benefits. Also, methodological pitfalls are indicated in numerous clinical trials. To address this point, therapeutic compounds rely upon their ability to cross the B-CNS-B. The pervasive B-CNS-B impairment found in ALS likely impedes drug delivery to the CNS. Perivascular collagen IV accumulation in ALS patients, one distinct difference from animal models of ALS, could be hindering transport of therapeutics to the CNS. Similarly, drug therapy failures in AD clinical trials might be attributed to basement membrane collagen IV expansion. If pharmacokinetic issues are

confirmed, i.e. insufficient drug delivery to the CNS, then repair of the B-CNS-B might be an essential prerequisite to development of an effective therapy and collagen IV should be a primary therapeutic target. In summary, restoration of B-CNS-B integrity would foster delivery of therapeutics to the CNS as well as aid in removal of waste products from the CNS, providing an improved environment for motor neuron survival.

6. Five-year view

Effective therapy for ALS is an urgent need, yet developing treatment strategies for a multifactorial disease has proven extremely challenging. Despite intensive therapeutic development efforts, riluzole remains the only FDA approved ALS therapy and modestly extends patient lifespan.

The mismatch between effective drug therapies in animal model of ALS and unsuccessful drug treatments in ALS patients is disappointing and cause(s) of this discrepancy need to be identified and defeated. Animal models remain an essential tool for disease research but cannot be expected to provide a perfect window into human disease. Perhaps, an improved animal model of ALS, possibly primate, might facilitate effective therapy development for ALS in the near future. Also, increasing our knowledge regarding etiopathology of sporadic ALS should be addressed for identifying disease factors towards therapy development for all ALS cases.

Also, it is important to more deeply investigate pathogenic differences between animal models and human ALS. For example, differences in blood-CNS barrier alterations have been shown between sporadic ALS patients and animal models of disease. The specific barrier pathology found only in ALS patients might explain failed clinical trials in ALS. Blood-CNS barrier damage, a relatively recently discovered ALS pathology, may be a limiting factor in CNS drug delivery. The optimistic view is that: technological advances in the near future will allow non-invasive evaluation of barrier status in early stage ALS patients; effective therapies will be developed targeting barrier damage; and improved barrier status will promote the effective use of a variety of drug therapies for ALS.

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Reference annotations

* Of interest

** Of considerable interest

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7. Key issues

- There is a significant mismatch between results of pre-clinical studies (positive) and ALS clinical trials (negative)
- B-CNS-B is pervasively damaged in ALS patients
- Similarities and differences in B-CNS-B alterations have been shown in animal models of ALS and ALS patients
- Perivascular Collagen IV accumulation in ALS patients is one distinct difference from ALS animal models
- Basement membrane collagen IV buildup could hinder drug delivery across the blood-CNS-barrier
- Repair of the B-CNS-B might be an essential prerequisite to development of an effective therapy for ALS