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Review

Translating preclinical insights into effective human trials in ALS

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Abstract

Amyotrophic lateral sclerosis (ALS) is a rapidly progressive, adult-onset neurodegenerative disease characterized by selective dysfunction and death of motor neurons in the brain and spinal cord. The disease is typically fatal within 3–5 years of symptom onset. There is no known cure and only riluzole, which was approved by the FDA in 1996 for treatment of ALS, has shown some efficacy in humans. Preclinical insights from model systems continue to furnish ample therapeutic targets, however, translation into effective therapies for humans remains challenging. We present an overview of clinical trial methodology for ALS, including a summary rationale for target selection and challenges to ALS clinical research.

Keywords: Amyotrophic lateral sclerosis; Clinical trial; Preclinical model; Drug evaluation

1. Introduction

Because ALS is a uniformly fatal disease for which no adequate treatment exists, there is a pressing need to develop effective therapies. Preclinical research aimed at elucidating mechanisms of disease in large part specifies the kind of therapies that may be effective against motor neuron demise, and increasingly identifies specific candidate compounds for testing. Taking a drug from "bench-to-bedside" is an enormously costly and complex process. Here, we present an overview of important features in the discovery, development, and validation of disease-modifying therapies and interventions for ALS.

2. Rationale for target selection

2.1. Disease pathogenesis

The pathologic hallmark of ALS is the selective loss of motor neurons in the cortex, brainstem, and spinal cord. Determining molecular and cellular mechanisms that contextually favor motor neuron death in ALS is the starting point in identifying neuroprotective strategies. The pathogenesis of ALS is not completely understood and while much controversy remains about its molecular and biochemical biology, there is increasing consensus that multiple mechanisms of injury converge as the disease progresses and that caspase activation is a final pathway of cell death [1–3].

Important early insights into ALS pathogenesis resulted from a Paracelsian approach, in a phrase, that used the "patient as textbook" to learn about the disease. Studies in serum, cerebrospinal fluid (CSF), and post-mortem tissue implicated four principle mechanisms of injury: oxidative stress, excitotoxicity, axonal transport defects and protein mishandling. These pathways have been extensively explored in the context of cellular and/or animal systems used both to model disease and as major platforms for drug discovery. These model systems have provided further insights about potential contributors to disease including mitochondrial dysfunction, apoptosis, and inflammation.

2.2. Rationale for oxidative stress hypothesis

The hypothesis that oxidative damage is central to ALS was prompted by the discovery that mutations in a key cellular antioxidant, superoxide dismutase (SOD1), can cause ALS. While the majority of ALS cases are sporadic, about 10% of cases are inherited as an autosomal dominant trait and of these, 25% arise due to mutations in the gene encoding SOD1 [4].

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More than 100 mutations have been described to date [5] (for an updated list see the online database at www.alsod.org). Transgenic mice bearing point mutations found in humans, including the first and most widely studied SOD1G93A mutant [6], develop progressive motor neuron disease with clinical and pathologic features of the human disease [6–9]. The primary biochemical function of SOD1 is to convert superoxide, a toxic oxygen species generated by mitochondrial respiration, into hydrogen peroxide and water. While oxidative damage is an attractive proposal for the toxicity of mutant SOD1, it has been difficult to establish in experimental paradigms. SOD1 null mutant mice do not develop disease [10], and the level of enzyme activity is unchanged or elevated in mutant models that do develop disease [9], suggesting that diminished SOD1 activity does not cause motor neuron disease. Toxic copper-mediated chemistries involving aberrant substrates for mutant SOD1, including peroxynitrate [11,12] and hydrogen peroxide [13], have been proposed. Altering copper-loading onto SOD1 [14,15] or peroxynitrate precursors levels by manipulating nitric-oxide synthetase [16,17], however, had no effect on toxicity, suggesting that these aberrant reactions are not likely to have a major role in SOD-mediated disease.

Whether the primary toxicity of SOD1 mutants is oxidative remains uncertain. Some studies suggest that the toxicity of mutant SOD1 may relate to secondary effects of protein aggregates, including disruption of apoptotic regulatory mechanisms within cells (see below for discussion). Certain evidence, though, implicates oxidative injury in the pathogenesis of ALS. Studies of serum, CSF, and human post-mortem tissue have found increased markers of oxidative damage [18–21]. 8-hydroxydeoxyguanosine (8-OHDG), a marker of oxidative damage to DNA, is present in the blood and CSF of ALS patients and increases significantly with disease progression [22]. Clinical efforts to address oxidative damage currently include an early phase trial of the manganese porphyrin AEOL10150, which acts as a free-radical scavenger [103] (Table 1 lists all ongoing human trials).

The timing of oxidative stress in the disease process and the relevant cell type(s) responsible for elaborating reactive species are not known. There is biochemical and pathologic evidence, however, favoring involvement of this pathway in motor neuron dysfunction, and a variety of anti-oxidant compounds already exist. Thus, modulating oxidative stress remains a reasonably promising approach to neuroprotection and development of drugs to slow the progression of ALS.

Table 1
Current Clinical Trials in ALS

Compound	Mechanism	Clinical stage
Ceftriaxone	Anti-excitotoxic	Phase I–III
Minocycline	Anti-inflammatory, anti-apoptotic	Phase III
AEOL10150	Anti-oxidant	Phase I
Arimoclomol	Protein misfolding	Phase II
High-Dose Coenzyme Q	Mitochondrial dysfunction	Phase II
Sodium phenylbutyrate	Histone deacetylase inhibitor	Phase II
Ritonavir and hydroxyurea	Anti-apoptotic	Phase II
Thalidomide	Anti-apoptotic	Phase II

2.3. Rationale for glutamate hypothesis

The neurotransmitter glutamate can induce excitotoxic injury by accumulating at the synapse and causing repetitive depolarizations, cellular edema from sodium influx, and loss of calcium homeostasis in the post-synaptic neuron [23,24]. Compelling evidence for glutamate-mediated injury to motor neurons in ALS is the ability of riluzole to prolong survival in human trials [25,26]. Riluzole is thought to inhibit release of glutamic acid and act at the calcium-permeable NMDA receptor and voltage-gated sodium channels to attenuate cellular responses to glutamate. Defective glutamate handling in ALS was initially suspected based on markedly elevated levels of glutamate in the CSF of affected individuals [27–29] and the finding of impaired glutamate transport associated with loss of the EAAT2 transporter [30]. Five subtypes of glutamate transporters have been identified, but the astrocytic EAAT2 transporter buffers ~90% of the extracellular glutamate surrounding motor neurons [31,32]. Loss of astrocytic EAAT2 protein has been found in 60% of patients with sporadic ALS [31]. A mutation in the EAAT2 coding region detected in one patient with sporadic ALS was found to alter membrane targeting for the protein and impair glutamate uptake [33]. Interestingly, SOD1 mutants are associated with functional loss of EAAT2 in mice [34]. Because motor neurons possess a relative lack of calcium buffering proteins [35], and the configuration of the AMPA receptor on these cells confers increased permeability to calcium [36], motor neurons may be especially vulnerable to excitotoxic injury. Thus, targeting glutamate-mediated injury is a uniquely promising approach to drug development for ALS. Recently, Rothstein and colleagues performed an elegant screen of FDA-approved drugs in vitro to identify compounds that could up-regulate EAAT2 expression [37]. Many β-lactam antibiotics turned out to be potent inducers of EAAT2 expression, however, ceftriaxone showed the added benefit of increasing brain expression and activity of EAAT2 in animals and a demonstrated a clear neuroprotective effect in both in vitro and in vivo models of motor neuron disease. Whether ceftriaxone confers the same benefit in humans will be determined in an upcoming clinical trial in patients with ALS. Another approach would be to concentrate efforts on the sole drug that has proven efficacy against ALS. A better understanding of the drug–receptor interactions that mediate riluzole's beneficial effect may bring to light rational modifications to its structure that could improve efficacy. In all events, since a mechanism of injury can be described from a ligand–receptor interaction at the cell surface through multiple well-described downstream signaling cascades, the glutamate injury pathway offers a promising array of potential targets for therapeutic intervention.

2.4. Rationale for axonal dysfunction hypothesis

A pathologic hallmark of ALS is the accumulation of neurofilaments, abundant cytoskeletal components, in the soma and proximal axons of motor neurons [38]. In both human ALS and SOD1 transgenic animals, the largest caliber, most