

ALS CLINICAL TRIALS

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Most clinical trials are based on current hypotheses that have been developed to explain the pathogenesis of ALS. These hypotheses include mutations of copper/zinc superoxide dismutase (SOD1) in patients with familial ALS (FALS) that result in a toxic gain of function, glutamate excitotoxicity (impaired glutamate transporters), oxidative stress, neurofilament dysfunction, altered calcium homeostasis, impairment of neurotrophic factors, mitochondrial dysfunction, impaired cellular energy production, enhanced motor neuron apoptosis, proinflammatory cytokine activity, and microglial proliferation or inflammation [1-3].

Anti-Glutamate Strategies

Clinical trials of lamotrigine, branched chain amino acids, dextromethorphan, and more recently **gabapentin** [4] have all been negative. The recent topiramate trial showed no benefits [5]. Moreover, in this trial, the open-label phase was discontinued because patients receiving the drug had more pulmonary emboli, deep vein thrombosis, and renal calculi. Also, the patients taking topiramate developed greater muscle weakness and impaired pulmonary function, although survival did not differ from patients receiving placebo. A similar problem occurred in the ciliary neurotrophic factor (CNTF) clinical trial [6]. Careful and effective data safety monitoring is imperative to detect potential side effects as well as clinical benefits [7].

Ceftriaxone is a common "hit" found using various high-throughput technologies to identify potential compounds that may be neuroprotective for motor neurons [8]. It apparently activates EAAT2 expression [Dr. Jeff Rothstein, Johns Hopkins University, personal communication]. Based on these experimental studies, the NINDS has funded a randomized controlled trial (RCT, usually denoting a randomized, placebo-controlled clinical trial) for long-term (12 months) intravenous ceftriaxone treatment. A combined phase I and II study for long-term safety has been started (Dr. Merit Cudkowicz, NEALS Group).

Cannabinoid, delta(9)-tetrahydrocannabinol [delta(9)-THC], has anti-glutamate properties. In ALS mice, it delayed motor impairment and prolonged survival [9], and it has been proposed as a potential therapeutic agent for ALS.

Anti-Oxidant Strategies

A series of agents with antioxidant or neuroprotective properties or both has been studied in ALS, and include L-deprenyl, N-acetylcysteine, and calcium channel blockers, but so far none have been shown to be beneficial. A RCT of vitamin E taken orally at a dose of up to 1,000 mg per day also did not have a clinical benefit. Nonetheless, many patients take high doses of natural antioxidants, including various combinations of vitamin E, vitamin C, flavinoids, or all of these. Recently, an RCT to investigate the effects of high dose vitamin E (5,000 mg) for 18 months in patients who were also taking riluzole found no side effects but also no survival benefit [10].

Many patients with ALS are taking creatine, a naturally occurring muscle component important in mitochondrial function that is widely available as an over-the-counter supplement in health food stores. **Creatine** improves motor function in transgenic SOD1 animal models of ALS although there have been some conflicting results [11]. Recently, a well-designed RCT of high-dose (10 g) creatine in Holland had negative results based on survival as the primary endpoint [12]. A recent RCT in which patients received 5 g creatine daily with isometric muscle strength testing as the primary endpoint found no clinical benefits [13, 14]. A third study of creatine is in progress; change in fatigue is the primary endpoint (PI, Jeffrey Rosenfeld, MD, Carolinas ALS Center).

Edaravone is a potent free radical scavenger that is approved in Japan for the treatment of acute stroke. A small double-blind study has been performed in 40 patients with ALS in Japan [15]. Patients received either Edaravone 30 mg or placebo per day for 5 days for 4 weeks followed by an open-label study in which patients received the drug once a week for another 2 weeks. The study showed no improvement in ALSFRS-R score or FVC, but in a post-hoc analysis, those who had early ALS (defined as an initial high score on the ALSFRS-R scale) and received the drug had a significantly better ALSFRS-R score and FVC after treatment than those receiving placebo.

Manganese porphyrin (AEOL 10150), which is not an intrinsic biological compound, apparently is a potent anti-oxidant. The injection of this compound in ALS mice when they became symptomatic markedly prolonged survival (J. Crow, PhD; presented at the 14th International ALS/MND Symposium, Milan 2003; and Reference 16). This preclinical study in ALS mice is unique and important because the treatment was started at symptom onset and the benefits were dramatic compared to results of all previous studies. A phase I study to test the safety and feasibility of manganese porphyrin has been started at six ALS centers in the United States.

Co-enzyme Q10 is an anti-oxidant also thought to improve mitochondrial membrane and cellular energy production. Recent reports indicate that CoQ10 modestly improves disability in Parkinson's disease [17]. High doses (up to 3000 mg) of CoQ10 plus vitamin E (1500 mg) in patients with ALS are generally safe [18]. An NIH-funded multicenter phase II dose-determining study of high-dose CoQ10 (placebo, 2000 mg or 3000 mg) is scheduled to start in 2005 (PI, Dr. Petra Kaufmann, Columbia University).

Neurotrophic Factor Strategies

Neurotrophic factors are a heterogeneous group of cytokines that are produced in regulated amounts from various tissues and are important in cellular proliferation, differentiation, maintenance, maturation, and repair. A number of recombinant neurotrophic factors have been studied in well-designed RCTs, including **CNTF**, **brain derived neurotrophic factor (BDNF)**, **insulin-like growth factor (IGF-1, Myotrophin®)**, and **glial cell-derived neurotrophic factor (GDNF)**. Results from these trials have generally been disappointing [19]. It has been proposed that these negative findings may reflect poor drug delivery to target tissue and that future effort should focus on trying to mirror the natural biology of these and other neurotrophic factors more closely; systemic and intrathecal routes of administration do not appear to achieve adequate tissue levels and necessitate high doses that cause adverse side effects. The clinical benefit of **IGF-1** is being reinvestigated, this time by the Great Lakes ALS Study Group (PI, Eric Sorenson, MD). The primary endpoint is the change over time in the manual muscle strength testing score, whereas the primary endpoint in the original IGF-I trials was the change over time in the Appel ALS score. Patients are currently being enrolled.

Xaliproden, (Sanofi SR57746A) is a novel small peptide with both neurotrophic and neuroprotectant properties and good CNS penetration. Recently two RCTs (one with xaliproden and riluzole and the other with xaliproden alone) were designed to have two co-primary endpoints defined as: 1) time to death, tracheostomy, or permanent assisted ventilation (DTP), and 2) time to vital capacity (VC) of less than 50%. The drug demonstrated modest benefits for VC but not for DTP [20]. At this point, further clinical trials are unlikely.

Anti-Inflammatory Strategies

Evidence is increasing that proinflammatory cytokine levels are increased and microglia are activated in ALS patients and ALS mice [21-24]. A RCT with **celecoxib**, one of the cyclo-oxygenase-2 antagonists, was initiated by the Northeastern ALS Study Group but showed no difference in the primary endpoint, the change in the slope of isometric muscle strength measurements, between active medication and placebo [25].

Anti-Apoptotic Strategies

Evidence suggests that apoptotic pathways (caspase cascades) are activated in ALS [26-28]. Administration of **minocycline**, a caspase inhibitor and an anti-microglial cell activator, has shown moderate benefits in ALS mice in studies from various laboratories [29-31]. A safety and feasibility study demonstrated that minocycline was well tolerated, although gastrointestinal side effects did occur [32]. A large multicenter clinical trial is being conducted by the Western ALS Study Group and Columbia Study Coordinating Center (PI, Dr. Paul Gordon, Columbia University).

Anti-HIV medications have been reported to show intriguing benefits in atypical motor neuron diseases that are associated with HIV infection [33, 34]. **Indinavir**, a protease inhibitor for HIV infection that also acts as a lymphocyte anti-apoptotic antiviral agent, has been one of the principle medications used to treat HIV infection. Scelsa and colleagues [35] tested the benefits of indinavir in a pilot study in patients with sporadic ALS but no HIV infection. No clear benefits were found, although the number of patients was too small to make definitive conclusions. Patients who were on indinavir had more side effects such as nephrolithiasis and significant gastrointestinal symptoms. Because of such side effects, we do not believe further clinical studies are justified.

TCH346 (Novartis) binds glyceraldehyde-3-phosphate dehydrogenase and prevents p53-related neuronal apoptosis. TCH346 treatment in an animal (pnm mouse) model slowed disease onset and the clinical course [36]. A subsequent large multicenter, multinational study has been completed that was performed to investigate

the effects of TCH346 on the change in ALSFRS-R score over 9 months following a 4-month lead-in; results should be available at the time of this seminar.

Methyl-cobalamin is an old B12 derivative, but recently it has been found to have anti-apoptotic properties. Ultra high-dose intravenous methyl-cobalamin treatment for 4 weeks in patients with ALS improved compound motor action potentials on EMG [37]. A clinical trial is scheduled to start in Europe.

Other Neuroprotection Strategies

ONO-2506 is a glial cell modulating factor and appears to have neuroprotective effects via its action on glial cells. Its neuroprotective effects have been demonstrated in an animal model of brain ischemia [38]. An early RCT with ONO-2506 in ALS has recently been initiated in Europe. The results will be available soon.

Pentoxifylline increases cellular cyclic AMP and GMP, which are considered to function as neuroprotective agents in degenerating neurons [39]. However, a European phase II study to investigate its survival effects determined it exerted no beneficial effects [40].

Tamoxifen, a well-known anti-breast cancer agent, is a protein kinase C inhibitor. Anecdotally, in a patient with breast cancer and ALS who was treated with tamoxifen experienced marked slowing in progression of the ALS. Abnormally activated protein kinase C and abnormally phosphorylated proteins have been found in degenerating motor neurons. Based on promising preclinical experimental data in a retroviral model of motor neuron degeneration, the feasibility and safety of tamoxifen have been tested in both male and female patients with ALS. The drug was well tolerated, but both sexes experienced hot flashes [41]. In the same study population, data from an extended followup period suggested that patients receiving 20 to 40 mg per day may have longer survival compared to patients receiving only 10 mg per day (Dr. Ben Brooks, reported at the 15th International ALS/MND Symposium, Philadelphia, 2004). An RCT clearly is required.

The theory behind **hyperbaric oxygen** treatment in ALS is based on mitochondrial abnormalities found in ALS motor neurons. In a preclinical study in wobbler mice, treatment delayed onset of wobbler mouse motor neuron disease [42]. Bradley and colleagues tested hyperbaric oxygen treatment in 5 patients in an open design. Patients received hyperbaric 100% oxygen at 2 atmospheres for 60 minutes daily for 5 days a week for 4 weeks. Isometric muscle strength measurements revealed that strength markedly improved (up to 97%) in almost all muscles tested but that function did not improve. Most patients noted an improvement in fatigue, but one patient dropped out because of increased fatigue [43]. Further investigation is needed.

Neurovaccination Strategies

Vaccination regulates or suppresses inflammatory and non-inflammatory processes that damage tissues [44]. Vaccination strategies may be used to induce nonpathogenic T-cell responses, including activation of anti-inflammatory T_H2 cells [45]. T_H2 cells activated in response to vaccination may migrate toward a lesion site where they release inhibitory cytokines and neurotrophic factors [46, 47]. The inhibitory cytokines then curb local inflammation in an antigen-nonspecific manner. Glatiramer acetate (GA) is a mixture of synthetic polypeptides composed of four amino acids, L-alanine, L-glutamate, L-lysine and L-tyrosine in a molar ratio of 0.43: 0.14: 0.33: 0.1. It has an average molecular weight of 4,700 to 11,000 daltons. Vaccination with GA boosts anti-self T cell-mediated immunity without the risk of inducing autoimmune disease. It has been approved by the FDA for treatment of MS and delays disease progression in animal models of neurodegenerative disorders [48, 49]. Vaccination with GA generates regulatory T_H2 cells that may act in the CNS to suppress inflammation via the phenomenon of bystander suppression [45]. Evidence suggests that GA acts as a "universal antigen" containing multiple epitopes that induce proliferation and differentiation of naïve circulating T cells that can crossreact with a large variety of peptides. Thus, administration of GA induces highly crossreactive T_H2 cells that secrete T_H2 cytokines. When these T_H2 cells migrate to inflammation sites, they encounter self-antigens, which they recognize as weak agonists. The T_H2 cells then secrete anti-inflammatory cytokines and functionally active BDNF to restrict local inflammation and promote neuronal survival [47, 50].

In the MPTP model of Parkinson's disease, GA provided significant neuroprotection compared to placebo [51]. In this study, mice received adoptive transfer of splenocytes from GA- or ovalbumin-immunized mice. Adoptive transfer was used because MPTP immunotoxicity precluded active immunization studies. Pathologic analysis revealed that in those animals that received splenocytes from GA-immunized mice, T cells accumulated in inflamed areas in the CNS. T_H2 cells secreted IL-10, an inhibitory T_H2 cytokine, which suppressed microglial activation and also stimulated local expression of astrocyte-associated glial cell line-derived neurotrophic factor.

This immunization strategy minimized dopamine loss compared to control animals and significantly protected nigrostriatal neurons against MPTP-induced neurodegeneration.

This approach has been tested in ALS mice and demonstrated moderate benefits [48] but benefit was observed only in male mice [52]. We at Columbia University are interested in determining the safety and feasibility of chronic GA injections in patients with ALS. Thirty patients currently are enrolled in a single-blind study and receive GA injections either once a day or once every 2 weeks (PI, Dr. Paul Gordon, Columbia University). The patients' immunological status is also monitored.

Stem Cell Therapy

A small clinical trial with **allogeneic hematopoietic stem cell** (from HLA-matched siblings) transplantation in six patients has been reported by Appel and colleagues [53]. Two patients died, one progressed, two experienced a slowing of progression, and one patient had an unexpectedly stable course. Although in the central nervous system, 17% to 25% of total DNA was donor-derived, in the motor cortex, less than 1% was donor-derived DNA. Unusually high numbers of CD68+ cells were found in the CNS, suggesting a neuroinflammation induced by chemokine signaling.

In 2003, an Italian group reported their experience with **autologous cultured bone marrow mesenchymal stem cells** that were directly injected into the thoracic spinal cord in seven patients with ALS (Mazzini L et al; reported at the 14th International MND/ALS Symposium, Milan, Dec 2003). Embryonic or neurogenic stem cell research will require extensive basic research to establish preclinical feasibility studies in animal models before any human studies can be performed. A number of investigators have expressed serious concern about this type of stem cell research in human patients before the necessary basic research is done (Silani V et al. Autologous bone marrow stem-cell therapy and ALS. Letter submitted in response to Mazzini et al, Lancet).

Gene Therapy

Deletion of the survival motor neuron 1 (SMN1) gene has been found in spinal muscular atrophy (SMA) and conversion from SMN1 to SMN2 is associated with a milder form of SMA. Furthermore, the SMN gene has been reported to be a potential modifier in sporadic ALS: Corcia et al [54] reported abnormal copy numbers (1 or 3 copies) in the SMN1 gene in 16% of 167 ALS patients, but a smaller study (n = 50) found no such abnormalities [55]. Moulard et al [56] analyzed deletions in both telomeric and centromeric SMN gene DNA in 14 patients with pure lower motor neuron disease. Five patients had SMN centromeric deletions, suggesting that centromeric SMN deletion may be a susceptibility factor for pure lower motor neuron disease.

Andreassi et al [57] studied in vitro effects of **4-phenylbutyrate** (PBA), a histone deacetylase inhibitor, which increases gene activation. When fibroblast cell cultures from 16 patients with SMA of varying clinical severity were treated with PBA, in all cultures except one, PBA treatment increased the number of full-length SMN2 transcripts, ranging from 50% to 160% in cultures from patients with type I SMA and ranging from 80% to 400% in cultures from patients with type II or III SMA. PBA treatment also enhanced SMN protein levels and the number of SMN-containing nuclear structures. Patients with type II SMA (n = 10) tolerate PBA well and it appears efficacious; DNA studies of the SMN protein levels confirmed these findings [58]. Results indicate that PBA might be beneficial in patients with SMA without causing any major side effects. An RCT is underway (PI, Dr. Merit Cudkowicz, Massachusetts General Hospital).

Research into the use of viral vectors to deliver genes for ALS therapy is still in the preclinical stages. A study of **adeno-associated virus (AAV) transfer of the IGF-I gene** in ALS mice has had exciting results. When the genetically modified AAV was infected in the skeletal muscles of ALS mice after symptom onset, it resulted in remarkably prolonged survival and maintenance of motor function [59]. This preclinical study is particularly important because the treatment was started at symptom onset and the benefits were dramatic, far exceeding any other previous results. Dr. Jeff Rothstein, Johns Hopkins University, is currently exploring the possibility of beginning a phase I study in patients with ALS. Other gene therapy using siRNA or vascular endothelial growth factor has been tested in animal models [60, 61].

CLINICAL TRIALS FOR SYMPTOMATIC TREATMENT

The use of **dextromethorphan** is an important example of how an anecdotal clinical observation led to a successful clinical trial [62]. In early 1980s, dextromethorphan, which is an NMDA receptor antagonist and a sigma 1 receptor agonist (NMDA receptor modulator), was tried as a treatment for patients with ALS. The

investigators noted some improvement in pseudobulbar symptoms (uncontrolled laughter or crying). Now, almost 10 years later, a clinical trial of dextromethorphan, combined with quinidine sulfate (a metabolic inhibitor of dextromethorphan via inhibition of cytochrome P450) has been performed--- treatment significantly improved pseudobulbar affect in patients with ALS [63].

To reduce uncontrolled sialorrhea, the potential benefit of **botox injections** into the salivary glands is being investigated by Dr. Carlayne Jackson in an early safety and feasibility study.

In an RCT, Bourke and colleagues in the UK [64] studied the benefits of noninvasive positive-pressure ventilation (NIPPV) in patients with ALS. Twenty-two patients received NIPPV, and 19 had standard care. Patients with NIPPV survived significantly longer and had a better quality of life. NIPPV did not significantly benefit those who had severe bulbar palsy. A study of the effects of NIPPV and nutritional care has been funded by the NIH (PI, Dr. Edward Kasarskis, University of Kentucky). This project will start in spring 2005.

New Devices and Technologies

High-frequency chest wall oscillation is an airway-clearing method. The pulmonary assistive device (the Vest® system) used for such airway clearing has been the standard of care for children with cystic fibrosis and lung donors. Whether this device can improve the pulmonary status of patients with ALS is now being tested.

ISSUES FROM PREVIOUS ALS CLINICAL TRIALS

Without clinical trials, it is highly unlikely that any effective drugs will become available to treat ALS. Trials also may lead to discovery of the pathogenesis and cause of ALS. Moreover, clinical trials provide hope for patients and encourage positive attitudes in our patients. ALS is a relatively rare disease, and I believe that every patient, if he or she desires, should participate in a clinical trial. However, clinical trials in ALS have been most disappointing because, of more than 20 drugs tested in clinical trials to date, only one drug (riluzole) has received FDA approval. On the one hand, it is likely that no truly effective drugs have been tested in clinical trials; on the other hand, the trial designs may not have been sensitive enough to detect efficacy if the drug effect was small. Clinical trialists are responsible for developing the most effective and efficient clinical trial methodologies so that once potential drugs are developed, they are rapidly and accurately evaluated [65].

Lessons learned from the past

The history of RCTs in ALS is short. The following lists a number of issues that may have negatively affected clinical trial results [66]:

- Preclinical results in animal models have been unreliable in predicting the results of human studies, possibly because [67]
 - presymptomatic treatment strategies in animals may have been the wrong approach to take.
 - whether the SOD transgenic mouse represents human sporadic ALS is uncertain.
- Only few outcomes revealed potential benefits in ALS RCTs (survival, FVC, and the Appel ALS scale).
- The definition of death and therefore of survival has turned out to be more complex than clinical trialists had initially thought. With the advent of NIPPV and timing of tracheostomy, patients are kept alive longer, raising questions as to how to define death for RCTs.
- Variability in measurement techniques (not only due to intra-rater and inter-rater variability/reliability but also due to the inherent nature of subjective measurements) reduced study power.
- A large amount of missing data for quantitative measurements reduced study power. As their disease progresses, patients are less likely to undergo testing because visiting the study centers becomes too difficult, and the tests themselves become increasingly laborious and tiring.
- Expectations have been too high concerning the degree of benefit that would be seen (too great an effect size selected). Higher dosages may not always be better, and drug combinations (e.g., riluzole plus the study medication) may not be additive.
- Center effects and country effects may have jeopardized study results because of imbalance in enrollment and differences in treatment.
- When study designs were driven by cost constraints, this may have jeopardized study results (i.e., patient number and study duration met only the lowest needed levels).
- The "natural history" of the placebo group has been evolving, most likely due to improved overall care and management. Thus, using the natural history as a historical control presents significant difficulties in assessing the efficacy of a new drug.

- Phase II results were unreliable in predicting phase III outcomes for both beneficial effects and side effects.
- Changes in protocol from phase II to phase III studies (dosage levels and ratio of patients to be studied in active and placebo groups) may be a reason why results differed from what was expected in some studies.
- Co-primary endpoints may be a problem when only one outcome is positive.
- When no study medication is available to the participants after the trial, the dropout rate may increase. Also, pressure to enroll patients at study sites may cause potential problems because less stringent diagnostic criteria may have been applied.

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