Amyotrophic Lateral Sclerosis

ISSN: 1748-2968 (Print) 1471-180X (Online) Journal homepage: informahealthcare.com/journals/iafd19

THEME 6 THERAPEUTIC STRATEGIES

To cite this article: (2011) THEME 6 THERAPEUTIC STRATEGIES, Amyotrophic Lateral Sclerosis, 12:sup1, 113-118, DOI: 10.3109/17482968.2011.615524

To link to this article: https://doi.org/10.3109/17482968.2011.615524

Amyotrophic Lateral Sclerosis, 2011; 12(Suppl. 1): 113–118

informa
healthcare

THEME 6 THERAPEUTIC STRATEGIES

P92 THE ALSFRS @20: EVOLUTION OF THE ALSFRS-R, HISTORY, CLINIMETRIC PROPERTIES AND FUTURE DIRECTIONS

CEDARBAUM JM[1], MITSUMOTO H[2], PESTRONK A[3], RINGEL SP[4], FLORENCE J[3], SANJAK M[5], BROOKS BR[5]

[1]*Cytokinetics, Inc, South San Francisco, CA, USA,* [2]*Columbia University School of Medicine, New York, NY, USA,* [3]*Washington University School of Medicine, St. Louis, MO, USA,* [4]*University of Colorado Denver School of Medicine, Aurora, CO, USA,* [5]*Carolinas Medical Center, Charlotte, NC, USA*

Email address for correspondence: jcedarbaum@cytokinetics.com

Keywords: ALSFRS-R, clinical trials, history

Background: The ALS Functional Rating Scale was developed beginning in 1991 for use in clinical trials of new therapeutic agents for ALS. In the 20 years since its inception, the ALSFRS, now in its revised version as the ALSFRS-R, has become the most frequently used ADL assessment instrument in ALS trials, appearing in more than 175 peer-reviewed publications. The ALSFRS and ALSFRS-R have been applied in other patient management and research settings as well. The ALSFRS-R has become an accepted primary endpoint measure for Phase 3 clinical development.

Objectives: To review the history of the development and use of the ALSFRS and ALSFRS-R.

Methods: This presentation will review the concepts underlying the ALSFRS and ALSFRS-R, the clinimetric properties of the scales, and advances in their application over the last 20 years.

Results: The ALSFRS and ALSFRS-R are reliable and reproducible scales with good clinimetric properties. Their administration is easy to standardize, and it is easy for patients and caregivers to use. They have been shown to be effective in retrospective chart reviews of ALS patients. The ALSFRS and ALFRS-R have been validated for administration over the telephone, and may be administered by computer interface as well. The ALSFRS and ALSFRS-R have been translated into and validated in 8 languages, and have been used in clinical studies world-wide. The scales have been adopted in numerous types of research studies, including online registries, and patient-initiated social networking research projects. The rate of progression of ALSFRS-R from onset of disease has been confirmed to predict survival time in ALS patients. Versions of the ALSFRS have been developed for other neuromuscular diseases (IBMFRS) and for advanced stages of ALS. New methods of analysis have recently been proposed which have the potential to increase the validity of the scale in long-term clinical trials.

Discussion: The ALSFRS, as it has evolved into the ALSFRS-R, continues to be a valuable tool in ALS clinical and drug development research, and has become a template for assessing function in ALS and other neuromuscular diseases. Future evolution and application of the ALSFRS-R will be discussed.

P93 STATISTICAL MODELING TO ILLUSTRATE THE CONTRIBUTION OF AND EFFECTS OF DIFFERENTIAL MORTALITY AND FUNCTIONAL CHANGE ON JOINT RANK TEST OUTCOMES IN ALS

ARCHIBALD D[1], INGERSOLL E[1], MATHER J[1], SCHOENFELD D[3], KERR D[2], WANG Y-C[2], DONG Y[2], BOZIK M[1]

[1]*Knopp Biosciences LLC, Pittsburgh, PA, USA,* [2]*Biogen Idec Inc., Cambridge, MA, USA,* [3]*Harvard School of Public Health, Boston, MA, USA*

Email address for correspondence: eingersoll@knoppbio.com

Keywords: dexpramipexole, joint-rank test, ALSFRS-R

Background: The joint rank test (1,2) is a valid statistical approach to the analysis of functional outcomes adjusted for mortality, for which subjects are ranked on clinical outcomes (change in ALSFRS-R and death). The rankings generally result in deaths being ranked worse than survivors and ranked by time to death; survivors are then ranked by change in ALSFRS-R. Ranked scores are then compared between treatment groups using the Generalized Gehan-Wilcoxon rank test.

In a previously reported (3) Phase 2 study comparing dexpramipexole high dose (300 mg/day) versus low dose (50 mg/day) for 24 weeks in 92 ALS subjects, the high dose group showed a 68% reduction in the hazard of mortality ($p = 0.07$) and a 21% reduction in slope of decline of ALSFRS-R ($p = 0.18$). The slopes result alone probably underestimates the true treatment effect on ALSFRS-R due to the large imbalance in mortality favoring the high dose group. The joint rank test showed an estimated 28% improvement in mean rank ($p < 0.05$) for the high dose group.

Methods: For this abstract, statistical modeling simulations were used to evaluate how the joint rank test behaves under circumstances of concordance and discordance of hypothetical treatment effects on ALSFRS-R and mortality: equal effect on both; strong effect on ALSFRS-R but no effect or negative effect on mortality; and strong effect on mortality but no effect on ALSFRS-R.

Results: Results show that the joint rank test is more appropriate than the slopes analysis of function if there is a strong mortality effect and a moderate effect on function. If effects on mortality and function are similar, there is a modest loss of power with the joint rank test compared to the slopes analysis (due to the non-parametric ranking). However, the joint rank results may be more valid in that they are not dependent on questionable assumptions implicit in para-

ISSN 1748-2968 print/ISSN 1471-180X online
DOI: 10.3109/17482968.2011.615524

metric models. If there is a strong functional effect and a deleterious mortality effect, the joint rank test will appropriately diminish the level of significance compared to the slopes model. If there is a strong mortality effect and no functional effect, the joint rank test is not as powerful as a survival analysis.

Conclusions: These modeling results illustrate that the joint rank statistic is a reasonable and intuitively appealing primary analysis of function adjusted for mortality. The interpretation of the clinical significance of differences in mean joint rank scores strongly depends on analyses of the component ALSFRS-R and mortality data.

References

1 Ingersoll, *et al*. ALS 2010;11(Supplement 1):36.
2 Finkelstein, Schoenfeld, Stat Med 1999;18:1341–54.
3 Bozik, *et al*. ALS 2009;10(Supplement 1):28.

P94 NOVEL PHASE II TRIAL DESIGN: VARYING COMBINATIONS OF CONTEMPORARY AND HISTORICAL CONTROLS

MOORE DH, KATZ JS, MILLER RG

California Pacific Medical Center, San Francisco, CA, USA

Email address for correspondence: dmoore@cc.ucsf.edu

Keywords: clinical trials, design, ALSFRS-R

Background: The use of historical controls (HC) in phase II trials is controversial. However, an increasing number of small pharmaceutical companies with limited resources want to use HC for phase IIa trials. To optimize efficiency of trial design, and to mitigate concerns about HC, varying combinations of HC and contemporary controls might be considered.

Objectives: To evaluate varying numbers of contemporary and historical controls for comparison with treated subjects in a phase II trial.

Methods: We investigate a situation where resources limit total sample size to 60 patients. Using simulated data from placebo patients in 5 previous clinical trials, we compared reduction in ALSFRS-R slope over 6 months, that can be detected with 80% power in a variety of phase II designs:

DD1) 30 treated vs. 30 placebo, no HC
DD2) 30 treated vs. 30 placebo + HC
DD3) 50 treated vs. 10 placebo + HC
DD4) 60 treated vs HC

The comparison HC are matched (by entry symptom duration and initial FVC) to those in the trial. For designs with contemporary placebo plus HC (D2 and D3), a favorable outcome is either a significant ($p < 0.05$) difference between the contemporary patient groups (treated vs placebo) OR a significant difference between the treated and the combined contemporary placebo plus HC, after first testing whether contemporary placebo differ from HC.

Results: The minimum slope reduction that can be detected (with 80% power) decreases as we go from D1 to D4. The reduction must be 46% or greater for D1, 37% or greater for D2, 29% or greater for D3 and 27% or greater for D4. For designs D2 and D3, power is 57% (D2) and a 15% (D3) to detect a 30% change in the placebo slope.

Discussion: The most efficient design for detecting a slope reduction is the one where all 60 patients are treated and compared with matching HC (D4). However, this open label design carries the greatest risk of bias, and therefore, the lowest strength of evidence. The hybrid designs, D2 and D3, are less efficient but do provide some check on whether slopes are changing over time, possibly due to improved disease management. The 'conservative' design (D1) that makes no use of HC is the least efficient but may be preferred since its results, if favorable, could be used as one of the two 'registration' trials required for FDA approval.

Conclusion: Varying combinations of contemporary and historical placebos may be a novel way of improving the efficiency of ALS trials. A small contemporary placebo group, as part of a randomized, blinded trial, reduces the risk of bias and raises the strength of evidence compared with an open label trial utilizing HC.

P95 A MODEL TO EXPEDITE PHASE II CLINICAL TRIAL START UP IN AMYOTROPHIC LATERAL SCLEROSIS

SAWICKI D, YERRAMILLI-RAO P, FREEMAN M, CUDKOWICZ M

Massachusetts General Hospital, Boston, MA, USA

Email address for correspondence: dpulley@partners.org

Keywords: clinical trial research, collaboration, recruitment

Background: Delays in Amyotrophic Lateral Sclerosis (ALS) clinical trial start up are not cost effective and may prevent once eligible subjects from participating in a trial, lowering the potential subject pool for an orphaned disease. Riluzole is currently the sole FDA approved treatment for ALS, a disease for which the average life expectancy is 3-5 years. It is therefore imperative to advance clinical research in ALS free of logistical barriers.

We propose that the employment of a dedicated collaboration model will aid in accelerating clinical trial start-up processes compared with non-collaborative clinical trial submissions.

Objectives: The aim of this pilot study was to demonstrate that a dedicated collaboration with a phase II trial and all participating approving bodies in clinical research can, expedite start up time, improve trial efficiency and increase the projected enrollment period.

Methods: A pilot study was created in collaboration with a phase II trial center, the Contracts Office, and the Institutional Review Board (IRB) to expedite the review of time-sensitive therapeutic trial contracts and protocols. Real time data was collected on a phase II trial using the collaboration model and this was compared to two similar, previously conducted phase II trials that did not utilize this model.

The mean time in days of IRB and contract approval for two ALS phase II clinical trials was compared to the current phase II clinical trial using the collaboration model.

Results: Time from submission to IRB approval was 30 days using the collaboration model as compared to a mean time of 108 days for similar, previously conducted phase II ALS trials. Contractual Trial Agreement (CTA) execution was 28 days, as compared to a mean time of 105 days in previous clinical trials. Clinical research center (CRC) approval was obtained in 67 days. No data was collected in the prior studies on this particular time frame.

Discussion: This study shows that dedicated collaboration with a phase II trial management team and all approving bodies does accelerate start up processes. This acceleration was achieved without compromising trial integrity or forgoing good clinical practice. These results support the proposal to use a similar model for future clinical trials to improve study start-up and trial efficiency and increase potential enrollment.

P96 DATA MONITORING COMMITTEE (DMC) ORGANIZATION IN AN 18-MONTH AMYOTROPHIC LATERAL SCLEROSIS (ALS) SURVIVAL TRIAL: THE EXAMPLE OF A PHASE 3 TRIAL WITH OLESOXIME VS PLACEBO AS ADD-ON TO RILUZOLE

ABITBOL J-L[1], MEININGER V[2], CUDKOWICZ M[3], DE-RYCKE Y[4], CUVIER V[1]

[1]*Trophos, Marseille, France,* [2]*Hôpital de la Salpétrière, Paris, France,* [3]*Massachusets General Hospital, Boston, USA,* [4]*Institut Curie, Paris, France*

Email address for correspondence: jlabitbol@trophos.com

Keywords: olesoxime, safety, clinical trial

Background: There is an ethical and regulatory obligation to monitor safety in clinical trials. Several recent publications have shown that test treatment could negatively impact function or survival in ALS patients.

Objectives: To describe the organization and functioning of a DMC to ensure patient safety in a phase 3, 18-month ALS survival trial.

Methods: The DMC was composed of a neurologist (chair), a clinical pharmacologist and a biostatistician. Before the trial start the DMC convened with the sponsor to agree on a charter and decide on safety parameters: semi unblinded (treatment A and B) Kaplan-Meir survival curves, ALSFRS, Laboratory values, ECG parameters, adverse events (AE) rated as severe and all SAE reports. Quantitative variables were presented as cumulated distribution function curves per visits in addition to standard descriptive statistics or specific analyses according to guidelines (e.g., QTc). The safety report was prepared by an independent statistician using an automated process, extraction from the e-CRF database, analysis with R and production of the document with LaTeX. A blinded version of the safety report was provided to the sponsor and the trial steering committee. Two formal tests of worsening of survival were scheduled to be conducted when 50 and 100 events had occurred (total sample size N = 470, 150 events expected). Survival simulations were conducted to assess if trial prolongation could be required to record the expected number of events to ensure power of the study to detect treatment differences. The DMC holds closed meetings every 3 months and issues a recommendation to the trial steering committee to either continue as planned or advises otherwise.

Results: This DMC organization provides adequate way of ensuring patient safety. The interpretation of severe AEs was difficult due to the lack of online MedDRA coding. Survival simulations were conducted because the number of events appeared initially to be lower than expected with an exponential model. A Weibull model fits the data better and with no need for a trial prolongation.

Conclusion: This process provides a DMC with online and adequate safety information to allow patient protection. Training of neurologists in roles and responsibilities of being on a DMC should be encouraged.

P97 SUCCESSFULLY TARGETING ALS/MND THERAPIES: THE DIAPHRAGM PACING EXAMPLE OF UTILIZATION OF THE FDA HUMANITARIAN PATHWAY FOR EXPEDIENT AND COST EFFECTIVE ACCESS TO NEW THERAPIES IN AN ORPHAN DISEASE

ONDERS R[1,2], IGNAGNI A[2], FRITZ M[2]

[1]*University Hospitals Case Medical Center, Cleveland, Ohio, USA,* [2]*Synapse Biomedical, Oberlin, Ohio, USA*

Email address for correspondence: raymond.onders@uhhospitals.org

Keywords: diaphragm, humanitarian, regulatory

Background: Diaphragm Pacing (DP) has been developed as a treatment for respiratory insufficiency in persons with ALS based on the successful use in high level spinal cord injury.(1,2, 3) The U.S. FDA Humanitarian Use Device (HUD) designation provides a regulatory pathway to bring devices to market in orphan diseases. Once designated as a HUD, evidence may be presented, to FDA, of safety and probable benefit for Humanitarian Device Exemption (HDE) market approval. DP has successfully navigated these pathways for ALS.

Objectives: Review the entire regulatory pathway to bring a novel new therapy for ALS/MND

Methods: Retrospective analysis of all of the regulatory pathways to commercialize DP.

Results: DP initial success in pure upper motor neuron (UMN) of SCI led to the initial FDA investigator initiated investigational device exemption (IDE) and IRB applications which began in 2003 with approval in 2004 for the pilot trial with first surgical implantation in 2005. Success of the pilot trial led to the FDA approved multi-center pivotal trial with first implantations in 2007. Prior to start of the trial the IDE was transferred to a commercial entity (Synapse Biomedical) and funding for the trial was raised through venture capital. The final one year patient follow-up occurred at the end of 2009 which allowed statistical analysis. There was a delay in obtaining HUD designation which needed a medically plausible subset of less than 4,000 US patients a year until an agreement was reached in 2010. HDE application was approved for probable benefit in ALS patients in 2011.

Discussion: The HDE pathway is rarely used in the U.S. compared to other means. In 2009 there were approximately 3,000 510(k) approvals, 15 original Pre-Market Approvals (PMA)'s and only 1 HDE approved. With the average total cost, from concept to approval, of a higher-risk PMA device of US$94 million; the return on investment for a company to develop devices in orphan diseases is severely limited.

Conclusions: DP is the first humanitarian device to be approved for ALS; it may also be the first device with an explicit indication for ALS. DP has been shown, using the HDE pathway, to be safe and of benefit in the treatment of respiratory insufficiency in ALS. The HDE pathway is a cost effective means to bring therapies to market for ALS patients. The success of DP will allow more commercial entities to invest in developing new therapies to improve the quality of life in patients with ALS/MND.

References
1. Onders RP, Calin AM, Elmo MJ, *et al*. Am J Surg 2009; 197:386–90.
2. Onders RP, Elmo M, Khansarinia S, *et al.* Surg Endosc 2009;23(7):1433–40
3. Onders RP, Khansarinia S, Weiser T, *et al.* Surgery 2010; 148:893–7

P98 PRELIMINARY EVALUATION OF THE IMPACT OF CONCOMITANT RILUZOLE ON DEXPRAMIPEXOLE TREATMENT EFFECTS IN ALS

ARCHIBALD D[1], INGERSOLL E[1], MATHER J[1], KERR D[2], CUDKOWICZ M[3], BOZIK M[1]

[1]*Knopp Biosciences LLC, Pittsburgh, PA, USA,* [2]*Biogen Idec Inc., Cambridge, MA, USA,* [3]*Massachusetts General Hospital, Boston, MA, USA*

Email address for correspondence: eingersoll@knoppbio.com

Keywords: dexpramipexole, ALSFRS-R, riluzole

Background and objectives: Dexpramipexole, a drug that may ameliorate mitochondrial dysfunction, is being developed to treat ALS. Riluzole is frequently used for ALS patients since its approval in 1996. The objective of this study is to report whether treatment with concomitant riluzole (non-randomized) modulates the treatment effects of dexpramipexole in a previously reported (1) small Phase 2 study.

Methods: The second part of this two-part, randomized, multicenter, double-blind, placebo-controlled study, was selected for analysis because power was increased with the greater per group sample size relative to part one. A total of 92 ALS patients were randomized to receive 50 or 300 mg/day dexpramipexole for 24 weeks. A total of 54 subjects were receiving concomitant riluzole (25 of 48 subjects in the 50 mg/day group; 29 of 44 subjects in the 300 mg/day group). Clinical outcomes were, in part, measured by the ALSFRS-R and survival. For this abstract, dexpramipexole treatment effects on functional decline and survival were examined in the subgroups of subjects receiving and not receiving concomitant riluzole.

Results: Subjects who received 300 mg dexpramipexole showed a trend towards a better ALSFRS-R slope (1.02) than subjects who received 50 mg (-1.28, $p = 0.18$), an improvement of 21%. The treatment effect of dexpramipexole on ALSFRS-R slope was similar in subjects receiving riluzole (-1.09 for 300 mg, -1.38 for 50 mg) and in subjects not receiving riluzole (-0.89 for 300 mg, -1.19 for 50 mg). There was no evidence of an interaction between dexpramipexole and riluzole on slope of ALSFRS-R ($p = 0.98$).

The 24-week mortality rate was lower in the dexpramipexole 300 mg group than in the 50 mg group, 7.2% vs 19.9%, which corresponds to a reduction in hazard rate of 68%. The effect of dexpramipexole on mortality was independent of concomitant riluzole use. In subjects taking concomitant riluzole, the Kaplan-Meier estimates (SE) were 17.2% (7.9%) in the 50 mg group and 7.1% (4.9%) in the 300 mg group. In subjects not taking concomitant riluzole, the estimates (SE) were 22.7% (8.9%) in the 50 mg group and 7.1% (6.9%) in the 300 mg group. The numbers of subjects in each cell (+/- dexpramipexole and +/- riluzole) were too small to warrant statistical evaluation of interaction effects.

Discussion and conclusions: In this small Phase 2 study, results suggest that concomitant riluzole treatment neither augments nor diminishes the treatment effect of dexpramipexole on survival or functional decline in ALS. It will be useful to confirm this observation in the definitive Phase 3 studies of dexpramipexole in ALS.

Reference
1. Bozik, *et al*. ALS 2009;10(supplement 1):28.

P99 FIRST IN HUMAN PHASE 1 TRIAL OF NEURAL PROGENITOR CELLS (NEURALSTEM) IN ALS: RESULTS IN THE FIRST 12 PATIENTS

GLASS J[1], BOULIS N[1], POLAK M[1], FEDERICI T[1], KELLY C[1], BORDEAU J[1], RUTKOVE S[3], HAZEL T[4], JOHE K[4], FELDMAN E[2]

[1]*Emory University, Atlanta, Georgia, USA,* [2]*University of Michigan, Ann Arbor, Michigan, USA,* [3]*Harvard University, Boston, Massachusettes, USA,* [4]*Neuralstem, Inc, Rockville, Maryland, USA*

Email address for correspondence: jglas03@emory.edu

Keywords: stem cells, human trial, spinal cord injections

We have completed 12 patient surgeries in this Phase 1 trial. Patients with ALS have been injected with fetal-derived neural stem cells into the lumbar spinal cord. The design is one of "escalating risk", where each group of patients are progressively less impaired. The first 6 patients were non-ambulatory, 3 of whom were supported by mechanical ventilation. The first 3 patients received 5 unilateral injections at L2-L4, and the next 3 received 5 injections bilaterally (total 10 injections) at the same levels. Patients 7-12 were ambulatory and had vital capacities > 60 % predicted. Patients 7-9 received 5 unilateral lumbar spinal cord injections, and patients 10-12 received bilateral lumbar injections. Each injection had a volume of 10 µl and a cell concentration of 10,000 cells/µl, so patients received either 500,000 or 1 million cells through 5 or 10 injections, respectively. The injection apparatus was fixed to the patient's spine, and so was able to move along with patient movement ("floating cannula") and avoid any lateral shear during the operation.

As of early May, 2011, there has been one death, unrelated to either ALS or the clinical trial. All patients tolerated the surgical procedure with minimal perioperative or postoperative problems. There have been no adverse events attributable to the cellular injections. Patients are immunosuppressed with a combination of tacrolimus and mycophenolate, which resulted in gastrointestinal distress in some patients.

The trial is continuing on schedule. We are using clinical evaluation, strength testing, and electrical impedence myography to monitor progression of disease. Following FDA review and approval of safety data from the first 12 patients we will move to injections into the cervical spinal cord. The conference presentation will provide up to date information on our progress to date.

P100 METALLOTHIONEIN DISPLAYS NEUROPROTECTIVE EFFECTS ON DORSAL ROOT GANGLION NEURONS AND EXTENDS SURVIVAL IN SOD1(G93A) MICE

LEWIS K[1,2], LEUNG YK[1,2], FOSTER D[1], BENNETT W[1,2], CHUAH I[1,2], CHUNG R[1,2], WEST A[1,2]

[1]*Menzies Research Institute Tasmania, Hobart, Tasmania, Australia,* [2]*University of Tasmania, Hobart, Tasmania, Australia*

Email address for correspondence: kealewis@utas.edu.au

Keywords: metallothionein, emtin

Background: The SOD1(G93A) transgenic mouse develops limb paralysis and spinal cord pathology that is similar to mutant SOD1-linked cases of ALS. Several studies have reported the upregulation of the neuroprotective metal-binding protein metallothionein (MT) in SOD1(G93A) mice and in ALS spinal cord. Additionally, SOD1(G93A) mice deficient in MT have a hastened disease endpoint.

Objective: In this study, we have used tissue culture to investigate whether MT is able to stimulate repair of injured axons. We have also investigated whether the neuroprotective effect of MT can be used advantageously to produce a better outcome for ALS.

Methods: In the *in vitro* experiments, explant or dissociated cultures of dorsal root ganglion (DRG) neurons from embryonic day 15 Sprague Dawley rats were maintained in Neurobasal medium containing 10% B-27 supplement. In some cases, dissociated DRG neurons were maintained in Campenot chambers, which allowed separation of cell bodies and processes, allowing precise evaluation of the effect of MT upon regeneration of injured DRG neurons. In the in vivo experiments MT was administered to SOD1(G93A) mice at 1mg/kg body weight twice weekly, via either unilateral or bilateral intramuscular injection. Mice received injections on one of two administration schedules: either commencing at 10 weeks of age and continuing until endpoint (during the symptomatic period); or for a period of 10 weeks commencing at 6 weeks of age (during the pre-symptomatic period). Motor impairment was assessed by hindlimb grip-strength, stride pattern and weight. Additionally, peptide analogues of MT ('emtins') were administered subcutaneously at 10mg/kg three times weekly from 12 weeks of age until endpoint.

Results: The results showed that MT can act neuroprotectively and stimulate nerve sprouting and regeneration of injured DRG neurons. The actions of MT involved interaction with the low-density lipoprotein (LRP) family of receptors, since a competitive LRP ligand (receptor associated protein) and the MAPK inhibitor PD98059 both blocked the actions of MT. Symptomatic intramuscular injection of MT resulted in an approximate 5% improvement in survival and a notable preservation in motor function. However, pre-symptomatic intramuscular injection of MT resulted in preservation of motor function but did not significantly increase survival. This indicates that MT may be acting directly on the peripheral nerves, or indirectly on the muscle, when injected intramuscularly throughout the symptomatic period. Symptomatic emtin peptide administration resulted in a similar improvement in survival, but did not appear to maintain motor function.

Discussion and conclusions: So far the results suggest that exogenous MT has a neuroprotective effect on injured nerves, possibly acting through LRP receptors. Administration of MT or emtin peptides may have a therapeutic benefit in SOD1(G93A) mice, which may be related to the neuroprotective effect of MT on peripheral nerves.

P101 THERAPEUTIC EFFECTS OF A FIRST-IN-CLASS THYROTROPIN-RELEASING HORMONE (TRH)-BASED LEAD COMPOUND IN THE G93A SOD1 TRANSGENIC MOUSE

COLE N, SLATOR G, HARDIMAN O, KELLY J

Trinity College Dublin, Dublin, Ireland

Email address for correspondence: kellyja@tcd.ie

Keywords: neurotherapeutic, TRH-based therapeutics, treatment

Background: Drugs that target multiple neuropathological mechanisms rather than a single mechanism may offer distinct therapeutic advantages in ALS treatment (1). Thyrotropin-releasing hormone (TRH) is a naturally-occurring neuropeptide with multifactorial neurotherapeutic effects relevant to ALS (2–4). Harnessing TRH's clinical potential has proved to be difficult because of its short half-life and potential neuroendocrine side effects (1–3). The ground-breaking first-in-class TRH-based lead compound (LC) we have developed overcomes these limitations and the weaknesses of existing TRH analogs. LC, a new chemical entity (patent application submitted), provides an innovative means to unlock the recognized medical potential of TRH-based neurotherapeutics in ALS treatment.

Objectives: To determine the ability of LC to provide therapeutic benefit in the G93A SOD1 transgenic mouse model of ALS.

Methods: Effects of LC in male G93A SOD1 transgenic familial ALS mice (high copy number; B6SJLTg (SOD1-G93A) 1Gur/J; The Jackson Laboratory, Bar Harbor, ME, USA) were investigated in a randomized, blinded, gender-weight- and age-matched study performed according to guidelines for preclinical animal research in ALS/MND. Treatment with LC (2 mg/kg i.p. 5 days/week) or vehicle was initiated in 2 randomly assigned groups ($n = 13$ per group) 6 weeks after birth and continued until end stage (death or when the mouse is unable to right itself within 30 seconds). Readouts were rotarod performance, body weight and survival.

Results: Mean rotarod performance was essentially constant until disease onset, which was observed to occur at 100 days, as indicated by a sudden 11% reduction in motor performance in both vehicle- and LC-treated mice. Following onset, motor function and mean body weight rapidly deteriorated in vehicle-treated animals. LC treatment consistently significantly improved rotarod performance and reduced weight loss after disease onset (two-way repeated measures ANOVA; $n = 13$, $p < 0.01$, Newman Keuls post-hoc test, for both readouts). Rotorod performance was increased by 21% at first measurement following onset (day 105) and increased to 109% on last day of measurement before first death (day 118); body weight was increased by an average of 6% over the same period. Median survival for LC-treated group was 132 days compared to 126 days for vehicle-treated animals.

Discussion and conclusions: Pre-symptomatic treatment with LC provides significant positive effects in two important measures of therapeutic benefit, namely motor function and weight. Data indicate for the first time that LC modifies the disease process in this ALS model and has potential to provide an innovative therapy for ALS patients.

References
1. Cavalli A, Bolognesi ML, Minarini A, *et al*. J Med Chem. 2008 Feb 14;51(3):347–72.
2. Kelly JA. Essays Biochem. 1995;30:133–49.
3. Gary KA, Sevarino KA, Yarbrough GG, *et al.* Pharmacol Exp Ther. 2003 May;305(2):410–6.
4. Reviewed in Ann N Y Acad Sci. 1989;553:388–472.

P102 ASC-J9 ENHANCES THE MUTANT TDP-43 DEGRADATION BY AUTOPHAGY

JIANG H, DUAN W, BU H, GUO Y, LI C

Department of Neurology, The Second Hospital of Hebei Medical University, Shijiazhuang, Hebei, China

Email address for correspondence: hblicy5@yahoo.com.cn

Keywords: autophagy, p62, TDP-43

Background: Mutations in the Cu,Zn-superoxide dismutase (SOD1) gene cause a familial form of amyotrophic lateral sclerosis (ALS) through an unknown gain-of-function mechanism. Mutant SOD1 aggregation may be the toxic property. Recently, 43-kDa TAR DNA-binding protein (TDP-43) was identified as the major disease protein found in ALS and frontal temporal lobar degeneration with ubiquitin-positive inclusions (FTLD-U). Mutant TDP-43 induced neuron damage, including apoptosis, oxidative stress, autophagy dysfunction and neurite outgrowth inhibition. P62 (also referred to as SQSTM1) is an adaptor scaffold protein, which binds both to polyubiquitinated proteins in aggregates and to LC3. P62 is one of the components of the ubiquitin-containing inclusions in several human neurodegenerative diseases. It plays important roles in forming inclusions and may be associated with the protection of neurons from degenerative processes.

Objective: To test natural products and their derivatives for selective degradation of misfolded proteins and protecting cells from the toxic effects of misfolded or mutated proteins.

Methods: To examine the solubility of TDP-43, sequential extractions were performed using RIPA buffer and urea buffer. P62 siRNA and mutant TDP-43 plasmids were transfected by using Lipofectamine following the manufacturer's instruction. The immuno-precipitation was carried out to detect the interaction of p62 and TDP-43. Western blot and Confocal microscopy analysis were used to test the expresson of TDP-43. TBARS in the cells and LDH content in the medium were tested.

Results: We found that 5-hydroxy-1,7-bis(3,4-dimethoxyphenyl)-1,4,6-heptatrien-3-one (ASC-J9) treatment up-regulated nrf2-dependent expression of p62, activated autophagy. It promoted p62-dependent degradation of mutant SOD1, mutant full length TDP-43 and the ~25, 35 kDa C-terminal fragment of TDP-43. But it had little effect on WT-TDP-43 degradation. The depletion of p62 decreased the recruitment of LC3 to mutant TDP-43, partly inhibited the degradation of mutant SOD1 and TDP-43. ASC-J9 also restored redistribution of endogenous TDP-43 from the cytoplasm to nucleus, activated Nrf2 and Nrf2 target genes and decreased oxidative damage. Interestingly, ASC-J9 promoted neurite outgrowth significantly.

Discussion: We found that autophagy contributes to the clearance of aberrant TDP-43 proteins. One main function of autophagy is to selectively eliminate toxic protein or damaged organelles. P62 can differentiate between WT and mutant toxic protein through levels of ubiquitination and increased the recruitment of LC3 to mutant protein. Enhancing the function of selective autophagy and increaseing the expression of p62 with drugs may be a tractable therapeutic strategy for associated diseases. ASC-J9 ameliorated the mutant protein-induced toxic effects by activating selective autophagy.

Conclusion: ASC-J9 may be a potential compound for neurodegenerative diseases.

P103 PROTECTIVE EFFECT OF DIMETHOXY CURCUMIN ON MITOCHONDRIAL DYSFUNCTION IN MUTATED HUMAN TDP-43 TRANSFECTED NSC34 CELL LINE

LU J, BU H, DUAN W, GUO Y, LI Z, LI C

Department of Neurology, The Second Hospital of Hebei Medical University, Shijiazhuang, China

Email address for correspondence: hblicy5@yahoo.com.cn

Keywords: dimethoxy curcumin, mitochondrial dysfunction, TDP-43

Background: Recently, one of the major disease proteins found in the pathological inclusions of amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration with ubiquitinated inclusions (FTLD-U) was identified as the TAR-DNA-binding protein of 43 kDa (TDP-43). Mitochondrial damage in TDP-43 linked animal and cell models have been reported. So we further studied the mitochandial function in our cell models.

Objectives: To further elucidate mitochondrial dysfunction in TDP-43 cell models, as well as to identify therapeutic approaches that target mitochondrial dysfunction and its consequences.

Methods: Mitochondrial morphology was tested by electron microscopy, mitochondrial complex I, and activity by spectrophotometric assays, mitochondrial member potential by flow cytometry and mitochondrial uncouple protein 2 expression level by Western Blot in four stably transfected cell lines. Then we observed the protective effect of dimethoxy curcumin (DMC) on mutated human TDP-43 cell line.

Result: Mutated human TDP-43 caused mitochondrial morphologic abnormality including mitochondrial swelling and disturbance of mitochondrial cristae, decreased complex activities, depolarized mitochondrial membrane potentials, and increased expression of mitochondrial uncoupling protein 2 (UCP2) in the NSC-34 cells. Meanwhile we found that the abnormal changes in mitochondria would be restored by DMC in mutated TDP-43 transfected cell lines.

Discussion: Studies on pathogenesis of TDP-43 are promising. Both gain and loss of TDP-43 functions are potential disease mechanisms. Mitochondrial dysfunction involves pathogenesis in ALS patients and in a variety of experimental models of SOD1-linked fALS. Our study showed that mitochondrial dysfunction exists in this TDP-43 cell model. Notably, DMC can ameliorate mitochondrial function in mutated TDP-43 transfected cell lines.

Conclusions: Mitochondrial dysfunction might be a common pathogenic mechanism in SOD1 related or TDP-43 related ALS. DMC could be a potential therapy approach in neurodegenerative diseases linked with mutated TDP-43.