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THEME 1 THERAPEUTIC STRATEGIES

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THEME 1 THERAPEUTIC STRATEGIES

P1 HUMAN ADIPOSE-DERIVED STEM CELLS ENHANCE THE GLUTAMATE UPTAKE FUNCTION OF GLT1 IN SOD1(G93A)

ZHANG C, GU R, HOU X, PAN R, CHENG F, GENG J, XU Y, LI L

Department of Neurology, First Affiliated Hospital, Sun Yat-Sen University, Guangzhou, Guangdong, China

Email address for correspondence: zhangch6@mail.sysu.edu.cn

Keywords: adipose-derived stem cells, glutamate transport 1, SOD1(G93A)

Background: Impaired glutamate uptake function of astrocytes associated with the accumulation of extracellular glutamate is a well-documented feature of amyotrophic lateral sclerosis (ALS). Enhancing the uptake function of astrocytic glutamate transport 1 (GLT1) may be a potential treatment for this disease. Human adipose-derived stem cells (hADSCs) are capable of secreting a large number of cytokines which exhibit diverse pharmacological effects.

Objective: To investigate the influence of the soluble factors released by hADSCs on the GLT1 in primary astrocytes cultured from SOD1 (G93A) mice, a widely studied mutant human SOD1 transgenic model of ALS.

Method: 1) Animals:Transgenic mice of the strain B6SJL-TgN (SOD1-G93A) 1GUR (No. 002726), were purchased from the Jackson Laboratory. 2)Isolation and culture of hADSCs: Human subcutaneous adipose tissue samples were collected from patients undergoing liposuction surgery after obtaining informed consent. 3) Primary spinal cord astrocytes culture: Primary mouse spinal cord astrocytes were prepared from postnatal day (P) 0 to P2 SOD1G93A mouse pups or matched non-transgenic littermates. 4) Human ADSCs and astrocytes co culture Transwell chambers with a 0.4 lm pore size membrane were used to physically separate the astrocytes from hADSCs. 5) Immunophenotype analysis: Flow cytometry (FCM) was used to analyze the immunophenotype of hADSCs. 6) Confirmation of multilineage differentiation of hADSCs: Human ADSCs were analyzed for their capacity to differentiate toward the adipogenic and osteogenic lineages. 7) Immunocytochemistry. 8) Western blot analysis. 9) Glutamate uptake assay. 10) Caspase-3 activity assay. 11) Quantitative real-time PCR assay. 12) Statistical analysis.

Results: Our data indicate that soluble factors from hADSCs significantly upregulate the expression of GLT1 in SOD1(G93A)-bearing astrocytes, which result in enhanced glutamate uptake function. The upregulation of GLT1 is accompanied by the inhibition of caspase-3 activation in mutant astrocytes. In addition, we find that hADSCs co cultured with SOD1 (G93A)-bearing astrocytes produce more VEGF, HGF and IGF-1, which are reported to have neuroprotective effects.

Conclusion: Our results suggest that hADSCs may be a potential candidate in cellular therapy for ALS.

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P2 INTRATHECAL TRANSPLANTATION OF HUMAN STEM CELLS IN TRANSGENIC AMYOTROPHIC LATERAL SCLEROSIS MOUSE MODEL

CHADI G, ALVES C, DE OLIVEIRA G, DUOBLES T, SCORISA J, BYDLOWSKI S, MAXIMINO J

Department of Neurology, University of São Paulo Medical School, São Paulo. SP, Brazil

Email address for correspondence: gerchadi@usp.br

Keywords: human stem cells, human amniotic fluid cells, human umbilical cord cells

Stem cell (SC) transplantation is a potential strategy for neurological diseases and SC research has expanded for Amyotrophic Lateral Sclerosis (ALS). Intrathecal injection is less invasive and is capable of extensively delivering cells by cerebrospinal fluid.

Human amniotic fluid (hAF) or human umbilical cord (hUC) Wharton's jelly-derived SCs, and vehicle as controls, were delivered intrathecally at the lumbar intervertebral space of $SOD1^{G93A}$ ALS mouse model (transgenic, TG), and also of wild-type (WT) mice, approximately 15 days before disease onset. Mice were followed behaviorally and spinal cord tissue was examined (histopathology and western blot) either at the onset or at the endpoint, as performed in separate groups of animals.

Bisbenzimide-positive SCs were found in the lumbar pial meninge and adjacent spinal cord white matter of SC-injected mice. hAF SCs, but not hUC cells, delayed disease onset by about 5 days, and also led to higher neurological scores and better hanging wire and rotarod performances throughout analysis. ChAT positive motoneuron counting in the mouse lumbar spinal cord ventral horn showed that hAF SCs fully counteracted and hUC SCs partially counteracted the cell number diminution found in the control TG vehicle mice in relation to WT already at the onset of disease. However, such motoneuron protections disappeared at the endpoint. GFAP immunoreactive astroglial profiles increased in the anterior horn of the gray matter of all groups of ALS mice compared to WT at the onset and final stage of disease. In line with ChAT neuronal countings, the levels of ChAT protein were found to be maintained only in the hAF SCs group at the onset, as compared to WT, in contrast to the massive reductions found in the hAF SCs group at the final stage and also in the hUC SCs at the two studied periods. Interestingly, hAF SCs fully counteracted the ability of TG mice to trigger astroglial activation at disease onset, but not at the end point, an event not found after hUC SCs treatment both at the onset and at endpoint as evaluated by means of GFAP immunoblotting. Moreover, OX-42 western blot showed that hUC SCs potentiated the ability of TG mice to trigger microglial activation at the disease onset, but not at the endpoint. hUC SCs injection increased the protein levels of 21 and 23 kDa FGF-2 isoforms at the onset compared to WT group, however 23 kDa isoform was found to be elevated in the hUC SCs group compared to

hAF treatment. A single intratecal lumbar injection of hAF SCs may have promoted transient behavior and motor neuron protections in the ALS mouse model, events that may be related to the ability of hAF cells to counteract astroglial activation in early phases of disease. Support: FAPESP, CNPq, CAPES

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P3 ASSESSMENT OF HUMAN FETAL NEURAL STEM CELLS IN THE ALS TRANSGENIC MOUSE MODEL

KNIPPENBERG S[1], RATH KJ[1], THAU N[1,3], DENGLER R[1,3], SCHWARZ J[2], WEGNER F[1,3], PETRI S[1,3]

[1]*Hannover Medical School, Hannover, Germany,* [2]*University of Leipzig, Leipzig, Germany,* [3]*Centre for Systems Neuroscience, Hannover, Germany*

Email address for correspondence: Knippenberg.Sarah@mh-hannover.de

Keywords: SOD1 (G93A) mice, neural stem cells, survival study

Background: Administration of stem- or progenitor cells is currently being investigated as novel therapeutic option in ALS and other neurodegenerative diseases. Several studies have already shown neuroprotective potential of several types of adult stem cells which were mainly attributed to their release of trophic factors. Due to their differentiation potential, neural stem cells of fetal origin are of particular interest for the therapy of neurological disorders.

Objectives: We evaluated the effects of direct intraspinal administration of human fetal neural stem cells (NSC) in the G93A mouse model of ALS at presymptomatic disease stage.

Methods: Human neural progenitor cells were derived from CNS tissue of aborted human fetuses (gestational week 10–18) with mother's consent. All experiments were approved by the Ethics Committee of the University of Leipzig, Germany and in accordance with all state and federal guidelines. One week before surgery, NSC were thawed and expanded at standard conditions in a reduced atmosphere (3% O_2). On the day of surgery they were diluted in sodium chloride to a volume of 100,000 cells per 1µl. The animals received 1µl bilaterally into the lumbar region of the spinal cord. Control animals received 0.9% sodium chloride as vehicle. Groups were further divided into a survival group and a group of animals that were sacrified at day 110 for immunohistological analyses. Animals of the survival groups were monitored for survival, general condition, weight and motor function (rotarod and footprint analyses). Additional animals were injected with GFP-labeled NSC and sacrified at different time points to monitor survival, migration and differentiation of injected cells.

Results: Intraspinal surgery was well tolerated by all animals. Significant improvement of NSC-treated mice was detected in motor performance tests and general condition scores. Survival analysis is still in progress. One week after surgery, GFP-labeled NSC were found along the puncture channel in the spinal cord tissue. Analysis of further time-points (2, 3, 4 and 6 weeks) as well as further histological analyses (co-localization of GFP-positive cells with neural and glial markers, motor neuron counts, quantitative analysis of astrocytosis and microgliosis) is still ongoing.

Discussion and conclusions: Intraspinal stem cell injection was shown to be safe and well tolerated in ALS transgenic mice and has already been shown to be feasible in ALS patients. In our study, we found positive effects of lumbar NSC-injection on motor performance and general condition. Further analysis needs to determine how long transplanted cells can survive in the spinal cord and what exactly underlies their beneficial effects. Possibly, repeated injections at more than one injection site will be necessary to maximize the protective potential of adult stem cells in ALS.

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P4 TRANSDUCTION OF CORTICOSPINAL MOTOR NEURONS BY AAV2 FOLLOWING DIRECT INJECTION INTO THE MOTOR CORTEX

VILLA S[1], JARA J[1], BOHN M[2,4], OZDINLER H[1,3]

[1]*Davee Department of Neurology,* [2]*Department of Molecular. Pharmacological and Biological Chemistry,* [3]*Cognitive Neurology and Alzheimer Disease Center; Lurie Cancer Center., Northwestern University Feinberg School of Medicine, Chicago, IL, USA,* [4]*Department of Pediatrics, Children's Memorial Research Center, Chicago, IL, USA*

Email address for correspondence: srvilla@u.northwestern.edu

Keywords: gene therapy, corticospinal motor neurons, AAV

Background: The use of adeno-associated virus (AAV) in gene therapy has multiple advantages due to its long-term expression in the central nervous system (CNS) and low immunoreactivity in humans. AAV-mediated gene therapy strategies have been considered for neurodegenerative diseases such as Canavan's disease, Alzheimer's disease and recently, motor neuron diseases including amyotrophic lateral sclerosis (ALS). AAV-IGF increased the lifespan of the well-characterized $hSOD1^{G93A}$ ALS mouse model. AAV serotypes come in many "flavors" given by capsid engineering to improve transduction efficiency and cellular specificity. Targeting only specific neuron population without affecting other neuron types within the cerebral cortex represents a major obstacle for translational neuroscience.

Objectives: In this study, we investigated the specific tropism of AAV serotypes for corticospinal motor neurons (CSMN). Seven AAV serotypes (AAV2-1, AAV2-2, AAV2-5, AAV2-6, AAV2-7, AAV2-8, and AAV2-9) that harbor the eGFP gene were tested after direct injection into the layer V of the motor cortex. CSMN transduction was confirmed by CTIP2 expression and by retrograde labeling with red fluorescent microspheres injected into the corticospinal tract (CST). In addition, using immunocytochemistry approaches we determine other cell types transduced by AAV serotypes including neurons (NeuN), callosal projection neurons (SATB2), astrocytes (GFAP), microglia (Iba-1), and oligodendrocytes (OLIG2).

Results: We find that different AAV serotypes have varied tropism for different neural cell populations in the motor cortex. For example, AAV 2-1 and AAV2-9 transduce a mixed cell population, including astrocytes. AAV2-5 exhibits tropis for callosal projection neurons, whereas AAV2-2, AAV2-6, and AAV2-8 show relatively high tropism for CSMN. However, none of the serotypes examined have selective tropism for CSMN.

Discussion: We have previously reported that CSMN are specifically transduced by AAV following microinjection into the corticospinal tract that lies within the dorsal funiculus of

the mouse spinal cord. However, anatomical differences between the mouse and human motor neuron circuitry may hinder translation of this method to a therapeutic context for gene therapy in humans. Therefore, identification of AAV serotypes that can transduce specific neuron populations upon direct cortical injection would be invaluable for potential gene therapy approaches, and for building effective treatment strategies for ALS patients.

Conclusions: Here we report that AAV2-2, AAV2-6, and AAV2-8 exhibit tropism for large pyramidal neurons in layer V of the motor cortex, including CSMN. Further enhancement of their tropism specifically for CSMN by engineering elements of the capsid proteins is an attractive and promising avenue of future research.

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P5 RESETTING INTRACELLULAR PH AS A POTENTIAL THERAPEUTIC APPROACH FOR AMYOTROPHIC LATERAL SCLEROSIS TESTED IN G93A MUTANT MICE

JIANG M[1], SCHUSTER J[2], KUO S-W[1], HECKMAN CJ[1]

[1]*Northwestern University, Chicago, IL, USA,* [2]*Seattle Children's Hospital, Seattle, WA, USA*

Email address for correspondence: m-jiang@northwestern.edu

Keywords: intracellular pH, motoneuron degeneration, mSOD1 mouse

Background: Studies on neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS), have revealed several well-defined pathogenic processes such as excitotoxicity, reactive oxygen species (ROS) and apoptosis. It is also becoming clear that these pathogenic processes may lead to intracellular acidification that in turn accelerates pathological progress of the diseases. While the efforts in preventing and treating the neurodegenerative diseases have produced limited success, much less attention has been made to work on homeostasis of intracellular pH (pHi) in these diseases, and none has been done on ALS to our knowledge. On the other hand, intracellular alkalinization has been implicated in preventing cell death. We thus hypothesize an alkalic shift of pHi in motoneurons will benefit survival of ALS patient.

Objectives: The objective of this investigation is to evaluate therapeutic potential of resetting pHi using ALS mouse model (mutant G93A SOD1, mSOD1) by either acidification/alkalinization shifting of pHi, or in combination with riluzole. We focus on the effects of pHi on survival, behavior and spinal motor function ALS animal model.

Methods: Female adult mSOD1 mice were divided into four groups treated with NH_4Cl, $NaHCO_3$, and $NaHCO_3$ plus riluzole in various combinations starting at age of 85 postnatal days. The end stage of the mSOD1 mice were determined by failure on a rotarod for less than 5 seconds examined on daily basis. The progress of the disease was evaluated by monitoring hindlimb tremor, muscle weakness and body weight once a week. And spinal motor function was evaluated with *in vitro* root reflex technique.

Results: The main finding is that $NaHCO_3$ treatment significantly prolonged the end stages of the mSOD1 mice (the end stage for control mice: 136.0 ± 10 days, mean $\pm$ SD, $n = 39$; for $NaHCO_3$: 149.6 ± 9.6 days, mean $\pm$ SD, $n = 12$; $p = 0.008$), appearing as a right shift in Kaplan-Meier survival (KMS) plot. Although no significance was found for NH4Cl treatment (133.4 ± 4.1 days, mean $\pm$ SD, $n = 12$), the pattern of its KMS plot appeared different from control, suggesting a more complicated process in acidification treatment.

Discussion: PHi value is known to be affected by extracellular pH. Since motoneurons in ALS degenerate due to pathological changes mostly occurred inside their cell body, the prolonged survival of mSOD1 mice treated with $NaHCO_3$ is likely achieved through the change of pHi. In addition, NH4Cl was reported to cause pHi alkalinization in acute *in vitro* study while its chronic application at system level was found to acidify the body, which may explain the special pattern for its KMS plot.

Conclusion: The data suggest direct alkalinization of pHi improves the survival of mSOD1 and deserves further investigation as a new therapeutic strategy.

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P6 ACTH (ACTHAR GEL): PRECLINICAL STUDIES IN THE G93A-SOD1 MOUSE MODEL OF ALS

ARRAT H, SIDDIQUE T, LUKAS T

Northwestern University, Chicago, IL, USA

Email address for correspondence: t-lukas@northwestern.edu

Keywords: adrenal corticotrophic hormone, preclinical, drug

Background: There is no effective treatment or cure for amyotrophic lateral sclerosis (ALS), where survival after diagnosis is usually less than 5 years**.** One of the common features of both sporadic and familial disease is that neuroinflammatory processes are evident with disease onset or shortly thereafter. ACTH (adrenal corticotropic hormone) has anti-inflammatory, neurotropic and myotropic effects, but has not yet been tested in an ALS mouse model.

Objectives: 1: Can ACTH be used as a therapy for neurodegenerative diseases like ALS? 2: What dose and route of administration (intramuscular (IM) or subcutaneous (SC)) provides favorable effects on disease onset/progression?

Methods: We used G93A SOD1 transgenic mice expressing a high copy number of the human gene, which develop ALS-like symptoms and pathology. All experiments were done under an approved ACUC protocol. ACTHar gel was provided by Questcor Pharmaceuticals (Anaheim Hills, CA). The preparation contains the active hormone (ACTH1-39) in a gelatin matrix (16%) at 400 U/mL. Mice were given the drug at different doses (120 U, 60 U/kg) by IM or SC routes either on an every other day basis or on a weekly regiment. Each arm of the trial had 5–10 animals and a separate control group of 20 animals who were given gelatin. We began drug administration in presymptomatic 60-day old animals and continued until end-stage. The mice were monitored for clinical symptoms such as tremor and hind limb paralysis. Rotarod testing was also done on a weekly schedule.

Results: The administration of ACTHar gel was well tolerated. Even with a fairly large dose (120 U/kg) the mice exhibited no acute side effects. All SC dose regiments were found to delay onset of disease by 12 to 19 days (log rank $P = 0.0001$. Onset of paralysis was also delayed by 6–8 days (log rank $p = 0.05$). However, survival was not extended significantly, (log rank $p = 0.313$) for any arm of the study.

Rotarod performance for all SC-treated animals improved significantly within a 4–5 week window (Weeks 9–14), $p < 0.01$ for males and 0.001 for female mice. Finally, the levels of G93A-SOD1 in the treated animal spinal cords were reduced by at least 75% ($p = 0.001$–0.01).

Discussion: ACTHar is FDA approved for treating relapsing multiple sclerosis, infant spasms, and nephritic syndromes. The tolerance to the drug and the significant effects on disease onset and early progression suggests that ACTHar gel has potential as a therapy for ALS. The surprising decrease in levels of SOD1 may add an additional benefit for patients with familial SOD1-linked disease.

Conclusions: Additional studies are in progress to optimize the dose of ACTHar gel to enhance survival as well as delayed onset and early progression.

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P7 DELETION OF THE BH3-ONLY PROTEIN BID INHIBITS ASTROCYTE AND MICROGLIA ACTIVATION AND DELAYS DISEASE PROGRESSION IN ALS MICE

COUGHLAN K, BREEN B, BEHAN Á, KÖNIG H-G, CANNON S, WOODS I, KING M, STRASSER A, KIERAN D, PREHN J

The Royal College of Surgeons in Ireland, Dublin, Ireland

Email address for correspondence: karencoughlan@rcsi.ie

Keywords: astroglia, inflammation, BID

Background: ALS pathology is accompanied by glial cell activation and neuroinflammation. Increased levels of pro-inflammatory cytokines were proposed to mediate the neuroinflammation and resultant activation of apoptotic pathways via consecutive activation of death receptors, caspase-8 and the pro-apoptotic BH3-only protein BID.

Objectives: The aim of the current study was to examine the contribution of the BH3-only protein BID to motor neuron loss, ALS disease progression and pro-inflammatory signalling *in vitro* and *in vivo*.

Methods: RT-qPCR and Western blotting experiments were carried out on lysates from lumbar spinal cords to assess BID mRNA and protein levels across ALS disease progression in *SOD1*^{G93A} mice. BID deficient primary mixed motoneuron cultures were treated with pro-inflammatory cytokines to examine cytokine-induced signal transduction and cell death. KB-dependent reporter gene activation was monitored in transiently transfected mixed motorneuron cultures. We generated *bid*-deficient mice expressing human mutant *SOD1*^{G93A} and assessed for motor function, lifespan, and motoneuron survival ($n = 24$/group; age, gender (12 males/12 females), weight and litter-matched) in accordance with the most recent ALS guidelines for generating preclinical data (1)().

Results: *bid* mRNA levels were significantly increased across disease progression particularly at end stage and BID protein levels were significantly increased at PND 120. Interestingly, *bid* gene deletion protected primary mixed motoneuron cultures against interleukin-1β (IL-1 β) and interferon-γ (IFN- γ) mediated cell death. *In vivo*, genetic deletion of *bid* in *SOD1*^{G93A} transgenic mice delayed the onset and progression of the ALS phenotype. In addition, *bid* deficiency significantly increased motoneuron survival in *bid*$^{+/-}$ mice at end stage, and potently inhibited microglia and astrocyte activation. Reporter gene assays performed in mixed motoneuron cultures suggested that *bid* was indeed required for the efficient activation of the pro-inflammatory transcription factor nuclear factor-KB in response to pro-inflammatory cytokines, independent on its effect on cell death.

Discussions and conclusions: Our data suggest a dual role for BID as cell-death inducer and as an important mediator of inflammation and astrocytosis during ALS disease progression. These results are particularly interesting in view of recent findings (2) that suggested non-apoptotic roles of Bid in inflammation and innate immunity in colonocytes. In summary, our findings highlight BID as a possible therapeutic target in ALS.

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References

1. Ludolph *et al. Amyotrop Lat Scl*2010.
2. Yeretssian *et al.* Nature 2011;474:96–99.

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P8 THE NEUROPROTECTIVE COPPER-BIS(THIOSEMICARBAZONATO) COMPLEX, CUII(ATSM), INDUCES NRF2 ACTIVATION AND UPREGULATION OF ANTIOXIDANTS IN CULTURED ASTROCYTES: POTENTIAL MECHANISM OF ACTION IN TRANSGENIC MOUSE MODELS OF ALS

LIDDELL J[1], CROUCH P[1,2], WHITE A[1,2]

[1]*Department of Pathology;* [2]*Centre for Neuroscience; University of Melbourne, Parkville, Victoria, Australia*

Email address for correspondence: jliddell@unimelb.edu.au

Keywords: therapeutic, Nrf2, astrocyte

Background: We have shown that Cu^{II}(atsm) has therapeutic potential for the treatment of ALS. Cu^{II}(atsm) extends the lifespan, improves motor function and survival of motor neurons, decreases oxidative and nitrosative damage, and attenuates astrocyte and microglial activation in the SOD1G93A transgenic mouse model of ALS (1). Similar protective action has also been found in a SOD1G37R mouse model.

Objectives: This study seeks to elucidate the mechanism by which Cu^{II}(atsm) elicits these effects. As oxidative damage and astrocyte activation were attenuated by Cu^{II}(atsm), the potential stimulation of neuroprotective antioxidant systems of astrocytes was investigated in vitro.

Methods: Primary astrocytes cultured from mouse brains were treated with Cu^{II}(atsm) for up to 24 h. Biological activity of the compound was assessed by determining activation of signaling kinases by Western blot. Nrf2 is a predominantly glial transcription factor responsible for regulating antioxidant enzymes such as heme oxygenase-1 (HO-1) and

glutamate-cysteine ligase (GCL), the latter of which controls the synthesis of the critical antioxidant glutathione. Activation of Nrf2 was assessed by transfecting astrocytes with an antioxidant response element (ARE)-GFP reporter. Induction of the downstream Nrf2 targets HO1 and GCL were assessed by Western blot and activity assay, respectively. Glutathione content and export were also determined following Cu^{II}(atsm) treatment.

Results: Cu^{II}(atsm) caused phosphorylation of the signaling kinases Akt, ERK and JNK, indicating its biological activity. ARE-GFP fluorescence was increased with Cu^{II}(atsm) treatment, demonstrating activation of Nrf2. Cu^{II}(atsm) treatment also stimulated the downstream Nrf2 targets HO1 and GCL. Accordingly, glutathione content and export from astrocytes was also elevated by Cu^{II}(atsm) treatment. The latter is important, as glutathione export from astrocytes is essential to maintain the glutathione content of neurons.

Discussion and Conclusion: We have found that Cu^{II}(atsm) activates the transcription factor Nrf2 and upregulates the antioxidant systems of cultured astrocytes. This activation may contribute to the neuroprotective and disease-attenuating effects of Cu^{II}(atsm) observed *in vivo* in ALS model mice, and indicates that Nrf2 may be an important therapeutic target for the treatment of ALS.

Reference

1. Soon CPW, Donnelly PS, Turner BJ *et al.* J Biol Chem 2011;286:44035–44.

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P9 BROMOCRIPTINE RETARDS DISEASE PROGRESSION IN AN ALS MOUSE MODEL VIA SUPPRESSION OF GLIAL INFLAMMATION

TANAKA K[1], KANNO T[1,2], YANAGISAWA Y[2], YASUTAKE K[1,2], HADANO S[2], YOSHII F[3], IKEDA J-E[1,2]

[1]*Neugen Pharma Inc., Isehara, Kanagawa, Japan,* [2]*Department of Molecular Life Sciences,* [3]*Department of Neurology, Tokai University School of Medicine, Isehara, Kanagawa, Japan*

Email address for correspondence: kazu@neugenpharma.com

Keywords: oxidative stress, astrocytes, mutant SOD1 transgenic mice

Background: Recent studies have suggested that oxidative stress plays a critical role in the progression of motoneuron loss in amyotrophic lateral sclerosis (ALS). Thus, oxidative stress could be a main target for the development of novel therapeutic agents in ALS. Neuronal apoptosis inhibitory protein (NAIP) has been shown to suppress cell death induced by oxidative stress and to exert neuroprotective activity. Based on the NAIP function, we originally developed the NAIP-ELISA-based drug screening system and screened 953 neurotropic compounds containing medical drugs by using this system. Among hit compounds, a dopamine D2 receptor agonist bromocriptine (BRC), which selectively exerts cell protection against oxidative insults and has been used as a treatment for Parkinson's disease, was of interest.

Objectives: The aim of this study was to evaluate the efficacy of BRC in a congenic ALS-$SOD1^{H46R}$ mouse model.

Methods: Intraperitoneal daily administration of BRC (0mg, 1mg and 10mg/5mL/kg) was commenced after the onset of symptom in ALS-$SOD1^{H46R}$ mice (post-onset administration). To validate the BRC efficacy in ALS-$SOD1^{H46R}$ mice, we performed behavioral (balance beam, vertical pole, and footprint tests) and neuropathological analyses. To evaluate the additive effect of riluzole, mice were treated with vehicle or BRC in combination with riluzole, and motor performance was assessed by balance beam and footprint tests.

Results: Behavioral analyses revealed that the post-onset administration of BRC sustained motor function in ALS-$SOD1^{H46R}$ mice at a symptomatic stage (21–22 weeks of age) when compared with the age-matched vehicle-treated ALS-$SOD1^{H46R}$ mice. The BRC treatment prolonged 12% of post-onset survival interval in ALS-$SOD1^{H46R}$ mice compared with vehicle control (p, 0.05 by log-rank test). However, there was no synergistic or additive effect of riluzole on motor function and disease progression in ALS-$SOD1^{H46R}$ mice. We demonstrated that the BRC treatment delayed ChAT-positive neuron loss and reduced the levels of activated astrocytes and inflammatory factors, iNOS and TNF-α, in the anterior horn of spinal cord in ALS-$SOD1^{H46R}$ mice. *In vitro* studies showed that the BRC treatment reduced the level of extracellular TNF-α in lipopolysaccharide-exposured mouse astorcytes. The ALS-$SOD1^{H46R}$ mice treated with BRC also exhibited the reduced level of oxidative damage. Further, the BRC treatment upregulated several anti-oxidative stress factors such as HO-1 and ATF3 in SH-SY5Y cultured neuronal cells via a dopamine receptor-independent pathway.

Discussion and conclusions: Our present study demonstrated that the post-onset treatment of BRC sustained motor functions, suppressed glial inflammation, and retarded the disease progression after onset in the ALS-$SOD1^{H46R}$ mouse model, implying that BRC protects motoneurons from the oxidative insults via suppression of astrogliosis in ALS-$SOD1^{H46R}$ mice. Thus, BRC is a highly promising drug for ALS.

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P10 SLOWING DISEASE PROGRESSION IN THE SOD1 MOUSE MODEL OF ALS BY BLOCKING NEUREGULIN

SONG F[1,2], LIU J[1], RAVITS J[3], LOEB J[1,2]

[1]*Hiller ALS Clinic and Research Center, Department of Neurology,* [2]*Center for Molecular Medicine and Genetics, Wayne State University, Detroit, MI, USA,* [3]*ALS and Neuromuscular Translational Research University of California, San Diego, CA, USA*

Email address for correspondence: fsong@med.wayne.edu

Keywords: therapeutic target, neuregulin1, disease progression

Background: Neuregulin1 (NRG1) is both a membrane bound and secreted growth and differentiation factor that regulates glial development as well as survival, synaptogenesis, axoglial interactions, and microglial activation. We first reported aberrant NRG1 signaling in amyotrophic lateral sclerosis (ALS) (1). We have developed a targeted neuregulin antagonist called HBD-S-H4 (2) that given intrathecally reduces microglia activation in rat chronic spinal cord pain model (3). Therefore, here we hypothesize that blocking NRG1 with HBD-S-H4 could be a new potential therapeutic to slow disease progression in patients with ALS.

Objectives: We determined whether blocking NRG1 in the central nervous system slows disease progression and prolongs survival in the ALS-superoxide dismutase 1 (SOD1) mouse model.

Methods: The NRG1 antagonist HBD-S-H4 was delivered weekly through an implanted intracerebroventricular cannula for 9 weeks (started from 8 weeks of age at the preclinical stage to 16 weeks of age when disease onset showed in control ALS-SOD1 mice). Body weight, disease onset and progression, animal survival as well as pathological changes were measured in the HBD-S-H4 treated mice compared with saline-treated control group.

Results: We have recently shown that soluble forms of NRG1 are induced in human ALS and the SOD1 model and that NRG1 receptor activation on activated microglia is associated with disease progression. Our initial results show that weekly treatments for 9 weeks of recombinant HBD-S-H4 are not toxic, delayed disease onset, and prolonged survival in the SOD1 mice. Cellular pathological changes in HBD-S-H4-treated mice are underway.

Discussion and conclusions: We identified a common therapeutic target of NRG1 receptor activation on activated microglia in both ALS patients and ALS-SOD1 model. We are currently testing whether a NRG1 antagonist blocks microglial activation in the SOD1 model, as it does in other models and, whether this would be a potential therapeutic for patients with ALS.

References

1. Song F, Chiang P, Wang J, Ravits J, Loeb JA J Neuropathol Exp Neurol. 2012;71:104–15.
2. Ma Z, Li Q, An H, Pankonin MS, Wang J, Loeb JA J Biol Chem. 2009;284:32108–15.
3. Calvo M, Zhu N, Tsantoulas C *et al.* J Neurosci. 2010;30: 5437–50.

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P11 THERAPEUTIC EFFECT OF GENETICALLY MODIFIED MUSCLE PROGENITOR CELLS IN ALS MICE

DADON-NACHUM M[1], BEN-ZUR T[1], BARHUM Y[1], KUN D[1], BENKLER C[1], YAFFE D[2], OFFEN D[1]

[1]*Felsenstien Medical Research Center, Tel Aviv University, Tel Aviv, Israel,* [2]*Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot, Israel*

Email address for correspondence: chenbenk@post.tau.ac.il

Keywords: muscle progenitor cells, genetically modified cells, neurotrophic factors

Background: Neurotrophic factors (NTFs) preserved and protected motor neuron in ALS models. However, all the clinical studies with administration of NTFs in ALS patients failed. We have developed muscle progenitor cells (MPCs) populations expressing BDNF, GDNF, VEGF or IGF-1, (MPC-NTFs). Combined conditioned media collected from the cells rescued motor neuron cell lines (NSC-34) from various insults. Furthermore, MPC-NTFs transplantation enhanced the regeneration of rat sciatic nerves after injury. Here we examined the retrograde transport of NTFs along motor neuron axons and tested whether MPC-NTFs transplanted into muscles can improve the symptoms and survival of SOD1 mice.

Results: A mixture of MPC populations each expressing one of the four NTFs was transplanted into the hind legs of SOD1 mice on days 90, 104, 118 of life. We found a significant delay of symptoms (up to 30 days in the male) and extension of lifespan (12 days in the males and 18 days in females). However, transplantation of MPC alone or MPCs over expressing just GDNF did not elicit any improvement. The results suggest a synergistic effect of the transplantation of MPCs expression in several NTFs. In order to study the possible synergistic effect of the mixture of MPC-NTFs on a signal transduction pathway; we focused on the PI3K- AKT motor neuron survival pathway. We found that the supernatant of a mixture of condition media from MPC populations expressing NTFs increase the phosphorilated AKT by 6–8 folds compared to MPC expressing a single NTF.

Discussion and conclusions: Here we have built a novel powerful strategy that enables a stable, long-term administration of four NTFs cocktails. Since intramuscular inoculated muscle progenitor cells participate in the formation of post mitotic multinucleated fibers, this route of administration of genetically manipulated MPCs results in a stable, long-term expression of the four NTFs. The constant and continuous release of the critical NTFs from the muscle fibers through the neuromuscular junction into the motor neuron system, and the retrograde transport to the cell bodies in spinal cords probably inhibits death pathways. We hope that our study will lead to a novel strategy to slow the progress and alleviate the symptoms of ALS, and extend the life expectancy and quality of affected patients.

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P12 TREATMENT WITH PYM50028 IMPROVES NEUROMUSCULAR FUNCTION IN A MOUSE MODEL OF ALS

KALMAR B, DICK J, GREENSMITH L

UCL Institute of Neurology, London, UK

Email address for correspondence: b.kalmar@ucl.ac.uk

Keywords: muscle function, neuroprotection, motor units

Background: Mice expressing the mutant human SOD^{G93A} (mSOD1) have been extensively used to test drug candidates for ALS. In this study we examined the effects of a non-peptide neurotrophic factor inducer, PYM50028 (Phytopharm plc) on disease progression in mSOD1 mice. PYM50028 has been shown to be neuroprotective in a model of Parkinson's disease, where it induces the expression of BDNF and GDNF (1). Extracellular GDNF can prevent motoneuron degeneration (2). Therefore, trophic factor inducers that can increase endogenous GDNF production may be potential therapeutic candidates for ALS.

Objectives: To establish the effects of PYM50028 on neuromuscular function and motoneuron survival in mSOD1 mice.

Methods: The effects of PYM50028 in mSOD1 mice were compared to vehicle-treated mice, riluzole-treated mice, and mice treated with both PYM50028 and riluzole. There were 4 treatment groups (n = 15/group): 1) PYM50028; 2) Riluzole; 3) PYM50028 plus riluzole and 4) Vehicle. At 120 days of age mice underwent physiological assessment of hindlimb muscle force and motor unit survival. Motoneuron survival was assessed from fixed spinal cord sections whereas hindlimb

muscles (TA, EDL) were processed for histochemical analysis of succinate dehydrogenase (SDH) activity.

Results: There was a 40% improvement in hindlimb muscle force in 120 day old mSOD1 mice treated with PYM50028 compared to vehicle-treated mice. A similar improvement in muscle force was observed in mice treated with both PYM50028 and riluzole, although treatment with riluzole alone did not improve muscle force. In mice treated with PYM50028 alone or in combination with riluzole, there was a 10% and 15% increase in EDL motor unit survival, respectively. Treatment with riluzole alone failed to preserve any motor units. Furthermore, there was a significant increase in motoneuron survival in mice treated with PYM50028 alone or in combination with riluzole, so that, respectively, 40% and 50% more motoneurons survived than in vehicle-treated mice. Although riluzole had no detectable effects on muscle function, it did have a significant neuroprotective effect, and 30% more motoneurons survived in the riluzole only group than in the vehicle-treated group. SDH staining revealed extensive metabolic changes in 120 day old mSOD1 muscles which were not prevented by riluzole, but were almost completely prevented in the PYM50028-treated group.

Discussion: Our results show that PYM50028 improves muscle function and rescues motoneurons in mSOD1 mice. These effects are significantly greater than those of riluzole. The effects of PYM50028 in mSOD1 mice are not altered by co-treatment with riluzole. It is possible that the beneficial effects of PYM50028 in mSOD1 mice are due to its ability to induce the expression of neurotrophic factors.

References

1. Visanji NP *et al*. FASEB J 2008;22:2488–2497.
2. Brunet *et al*. J. Comp. Neurol. 2007;501(5):669–690.

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P13 DEVELOPMENT OF A C9ORF72 ALS ANTISENSE THERAPY AND A THERAPEUTIC BIOMARKER

DONNELLY C[1], OSTROW L[1], ZHANG P-W[1], MISTRY N[1], BALASUBRAMANIAN U[1], LI Y[1], TIENARI P[2], TRAYNOR B[3], SATTLER R[1], ROTHSTEIN J[1]

[1]*Johns Hopkins University School of Medicine, Baltimore, MD, USA,* [2]*Helsinki University Central Hospital, Helsinki, Finland,* [3]*National Institute on Aging, Bethesda, MD, USA*

Email address for correspondence: chrisdonnelly@jhmi.edu

Keywords: C9ORF72, antisense, iPS

Background: A hexanucleotide 'GGGGCC' repeat expansion in the noncoding region of the *C9ORF72* gene has recently been identified in ~30% of familial and ~4–10% of sporadic ALS cases and is therefore the most common genetic abnormality associated with ALS to date. Since the function of the C9ORF72 protein is unknown and a C9ORF72 rodent model has not yet been generated, few methodologies exist to begin to elucidate the pathogenicity behind this repeat expansion. However, repeat expansions in non-protein coding regions are the known cause of other neuromuscular disorders (eg, DM1/2, FXTAS) and pathogenicity is thought to arise by aberrant binding of *trans*-acting factors to the *cis* repetitive elements. This is perhaps best studied in DM1 where MBNL1 is sequestered by the 'CTG' expansion in the *DMPK* pre-mRNA resulting in altered gene expression and aberrant splicing.

Objectives: To characterize expression and splicing patterns due to the presence of a 'GGGGCC' repeat in the non-coding region of the C9ORF72 gene and utilize these data to develop a viable antisense oligonucleotide (ASO) therapy and therapeutic readout.

Methods: Generate using high-throughput screening, profile, C9ORF72 patient fibroblasts, iPS cells, iPS-differentiated motor neurons and astrocytes, and human autopsy tissue. Utilize the developed C9ORF72 cell lines and test multiple ASO sequences for C9ORF72 knockdown and normalization of the identified dysregulated/mis-spliced transcripts.

Results: We have identified expression patterns and splice variants unique to cells/tissue that contain the C9ORF72 repeat expansion. Moreover, we have been able to utilize C9ORF72 siRNA/ASO knockdown methodologies to normalize specific CNS genes dysregulated in the C9ORF72 transcriptome.

Discussion and conclusions: We have generated and profiled a number of ALS patient-derived fibroblasts, iPS cells, and iPS-differentiated astrocytes/neurons and have identified unique expression and splicing patterns in cells that carry the C9ORF72 repeat expansion. We have further validated these data using autopsy tissue from human C9ORF72 ALS patients. Since blocking the repeated *cis* elements by ASO and RNA knockdown application is thought to be viable therapeutic in other neuromuscular repeat expansions disorders (eg, DM1), we tested this possibility in patient-derived C9ORF72 cells. Using these techniques, we are able to knockdown C9ORF72 RNA and protein levels. Furthermore, our transcriptome profiling yielded a number of CNS-expressed gene targets, whose aberrant expression in C9ORF72 cells can be rescued with ASO/siRNA treatment suggestive of ideal biomarker candidates. These approaches will allow us to effectively develop 1) antisense-mediated therapeutic approaches to ALS and 2) a relevant pharmacodynamic readout for antisense efficacy.

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P14 TARGETING THE ENDOGENOUS HEAT SHOCK RESPONSE AS A THERAPEUTIC APPROACH IN SPINAL BULBAR MUSCULAR ATROPHY (SBMA)

GRAY A[1], MALIK B[1], NIRMALANANTHAN N[1], DICK J[1], LA SPADA A[2], HANNA M[1], GREENSMITH L[1]

[1]*UCL Institute of Neurology, London, UK,* [2]*UCSD, California, USA*

Email address for correspondence: a.gray@ucl.ac.uk

Keywords: animal model, Kennedy's Disease, motoneuron survival

Background: Spinal and bulbar muscular atrophy (SBMA), otherwise known as Kennedy's disease, is an X-linked, late-onset progressive neurodegenerative disease, which predominantly affects males. Pathologically, the disease is defined by selective loss of spinal and bulbar motoneurons with accompanying neuromuscular impairment. Molecularly, the disease results from an expansion in the CAG repeat in the androgen receptor (AR) gene which encodes a polyglutamine tract in the mature protein (1, 2). The polymorphic CAG repeat normally ranges from 9 to 36, but an expansion of greater than 38 repeats results in disease. Although the underlying pathophysiology of the disease remains largely unknown, it is related to abnormal nuclear

accumulation of the pathogenic AR protein. Several treatment strategies for SBMA have therefore focused on decreasing nuclear accumulation and protein misfolding. We have previously shown that treatment with arimoclomol, a pharmacological co-inducer of the heat shock response, reduces the formation of ubiquitinated inclusions, alleviates disease symptoms and increases lifespan in the $SOD1^{G93A}$ mouse model of ALS. In this study, we examined the effects of treatment with arimoclomol in a mouse model of SBMA in which mice carry 100 CAG repeats (AR100) in the human AR gene (3). These mice recapitulate typical hallmarks of the human disease, including motoneuron loss and accompanying neuromuscular deficits.

Objectives: To test the effects of arimoclomol on disease progression in SBMA mice.

Methods: Arimoclomol (120mg/kg/day) was administered orally (in drinking water) to male mice, after symptom onset, from 12 months of age. At 18 months, mice were anaesthetised with isoflurane and the distal tendons of the tibialis anterior (TA), extensor digitorum longus (EDL) and soleus hindlimb muscles cut and attached to force transducers. The sciatic nerve was exposed and stimulated to elicit muscle contraction. Muscle force, contractile characteristics and motor unit survival was determined in untreated and arimoclomol-treated AR100 and wildtype (WT) mice. Furthermore, the survival of spinal motoneurons was determined morphologically.

Results: Arimoclomol significantly improved hindlimb muscle force and contractile characteristics, rescued motor units and importantly, improved motoneuron survival in SBMA mice treated from 12–18 months of age.

Discussion: Upregulation of the heat shock response by treatment with arimoclomol after symptom onset may have therapeutic potential in the treatment of SBMA.

Conclusions: Since SBMA is a hereditary disorder, presymptomatic individuals carrying the mutation can be genetically identified. We are therefore currently investigating the effects of presymptomatic treatment of SBMA mice with arimoclomol (from 6 months of age), to establish whether this regime is more effective than that observed following treatment after symptom onset.

References

1. La Spada AR, Wilson EM, Lubahn DB *et al.* Letters to Nature 1991;352:77–79.
2. Fischbeck KH Brain Research Bulletin 2001;56:161–163.
3. Sopher BL, Thomas PS, LaFevre-Bernt MA *et al.* Neuron 2004;41:687–699.

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P15 SYSTEMIC ANGIOGENIN DELIVERY AS A THERAPY FOR PATIENTS WITH ALS

BEHAN Á, CANNON S, WOODS I, KIERAN D, PREHN J

Department of Physiology, Royal College of Surgeons in Ireland, Dublin, Ireland

Email address for correspondence: abehan@rcsi.ie

Keywords: angiogenin, therapeutics, SOD1 mouse

Background: A previous study has identified mutations in a gene encoding for angiogenin in ALS patients (1). Subsequent studies by our group demonstrated that angiogenin protects cultured motoneurons against ALS-associated, stress-induced cell death (2,3). Furthermore, we have demonstrated that systemic angiogenin protein delivery significantly increased life-span and improved motor function in SOD1G93A mice (3). These results also suggested that angiogenin protein delivery may be beneficial in treating patients with newly diagnosed ALS.

Objectives: The aim of the current study was to focus on developing these findings into a therapeutic technology based on the delivery of systemic angiogenin protein for the treatment of ALS and to investigate if angiogenesis is related to angiogenin's neuroprotection in ALS.

Methods: We examined the effect of systemic delivery of angiogenin on angiogenin serum levels and uptake in a mouse model of ALS, the SOD^{G93A} mouse. We also assessed whether angiogenin treatment increases angiogenesis and vascularisation in SOD^{G93A} mouse. We expanded the study to include a comprehensive dose-response investigation of the effect of systemic angiogenin protein delivery on life-span and disease progression and motor function in a post-symptom onset treatment paradigm in both SOD^{G93A} and TDP-43 mice ($n = 24$/group; age, gender (12 males/12 females), weight and litter-matched) in accordance with the most recent ALS guidelines for generating preclinical data (4).

Results: Our pharmacokinetic studies demonstrated a marked difference in angiogenin uptake and elimination in the SOD1G93A mice compared to their wild-type counterparts. Additionally, angiogenin uptake and angiogenesis was observed in the spinal cord of SOD1G93A mice following systemic administration of angiogenin. Dose-response studies demonstrate an extension in lifespan, an increase in motor function and motoneuron survival in mice models of ALS following systemic angiogenin treatment from our post-symptom onset treatment paradigm.

Conclusion: Together, our data suggest a role for angiogenesis the role of angiogenin and generate a comprehensive preclinical package for developing angiogenin as a therapy for clinical evaluation in ALS.

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References

1. Greenway *et al*, Nat. Genet. 2006.
2. Sebastia *et al*. Cell Death Differ. 2009.
3. Kieran *et al.* J Neurosci. 2008.
4. Ludolph *et al*. Amyotrop Lat Scl. 2010.

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P16 A MULTICENTER SCREENING TRIAL OF THE SAFETY AND EFFICACY OF RASAGILINE IN PEOPLE WITH ALS

WANG Y[1], MOORE D[2], KATZ J[2], SAPERSTEIN D[3], WALK D[4], SIMPSON E[5], GENGE A[6], BERTORINI T[7], FERNANDES J[8], SWENSON A[9], ELMAN L[10], SWERDLOW R[1], DIMACHKIE MM[1], McVEY A[1], HERBELIN L[1], MACCHI Z[1], BAROHN RJ[1], STUDY GROUP WALS RASAGILINE[1]

[1]*University of Kansas Medical Center, Kansas City, KS, USA,* [2]*California Pacific Medical Center, San Francisco, CA, USA,* [3]*Phoenix Neurological Associates, Phoenix, AZ, USA,* [4]*University of Minnesota, Minneapolis, MN, USA,* [5]*The Methodist Hospital System, Houston, TX, USA,* [6]*McGill University, Montreal, Quebec, Canada,* [7]*University of Tennessee, Memphis, TN, USA,* [8]*University of Nebraska Medical Center, Omaha, NE, USA,* [9]*University of Iowa Hospitals and Clinics, Iowa City, IA, USA,* [10]*University of Pennsylvania, Philadelphia, PA, USA*

Email address for correspondence: ywang@kumc.edu

Keywords: rasagiline, oxidative stress, mitochondrial dysfunction

Background: Despite multiple clinical trials and recent advances in understanding its pathogenesis, there is no cure or effective treatment for amyotrophic lateral sclerosis (ALS). Oxidative stress, mitochondrial dysfunction and apoptosis have been proposed as the cause of motor neuron death in ALS.

Rasagiline, a monoamine oxidase B inhibitor, is FDA-approved for the symptomatic treatment of Parkinson's disease. Rasagiline has demonstrated broad neuroprotective activities against a variety of neurotoxins in neuronal cell cultures and in the SOD mouse model of ALS. *in vitro* experiments indicate rasagiline stabilizes mitochondria under stress conditions.

Objectives: Specific Aim 1: To determine whether rasagiline is safe in this patient population and if the drug has the potential to slow ALS disease progression. Specific Aim 2: To determine if mitochondrial function is affected by rasagiline. We will measure and compare the change of BCL-2/BAX prior to, before and after the rasagiline treatment study drug.

Method: This is a phase II multi-center open label study in El Escorial probable or definite ALS who met our inclusion and exclusion criteria. Subjects are treated with rasagiline 2 mg daily for 12 months. The primary outcome measure is the change of the slope of ALS Functional Rating Scale-Revised (ALSFRS-R) over 12 months as compared with natural history data derived from four large completed ALS trials. The secondary outcome measure is the change in proposed biomarkers including blood leukocyte Bcl-2/Bax ratio, mitochondrial potentials in platelets, and indicators of apoptosis and oxidative stress following rasagiline administration. We are also evaluating safety laboratory and clinical data.

Results: Nine centers in the Western ALS (WALS) study group are participating in this study. These centers recently completed enrollment of thirty-five ALS patients. There were 18 men and 17 women, with a mean age at entry of 61 years (59–82), and a mean disease duration of 1.5 years (0.5–3). The mean ALSFRS-R at study entry was 38.6 (31–45). Currently, rasagiline 2 mg is safe and well tolerated in patients with ALS. However, four patients did drop out due to side effects (dizziness) of the medication and there was one patient death unrelated to the study drug.

Conclusion: Six month data on primary and secondary outcome measures as well as safety data will be available before the meeting and will be presented.

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P17 DEXPRAMIPEXOLE IS NOT CONVERTED TO PRAMIPEXOLE AFTER ADMINISTRATION IN HUMANS

WEI D, WU C, HE P, KERR D, STECHER S, YANG L

Biogen Idec, Cambridge, MA, USA

Email address for correspondence: dong.wei@biogenidec.com

Keywords: dexpramipexole, pramipexole, chiral interconversion

Background: Dexpramipexole (DEX) is currently being investigated in clinical studies for the treatment of amyotrophic lateral sclerosis (ALS). DEX is the R-(+) enantiomer of pramipexole (PPX), which is a non-ergoline dopamine agonist marketed (Mirapex®) for the treatment of Parkinson's disease and restless legs syndrome. Although DEX and PPX may share neuroprotective properties, DEX is pharmacologically distinct from PPX in that DEX has minimal affinity for dopaminergic receptors.

Objective: This study was designed to determine whether PPX can be identified in the circulation at pharmacologically significant levels following oral administration of DEX in humans.

Methods: Healthy adult subjects were administered doses of DEX up to 600 mg. A plasma sample was taken 2 hours after dosing. To monitor potential circulating PPX, a highly sensitive and selective chiral LC-MS/MS assay was developed and qualified for the detection of PPX in the presence of DEX in human plasma. In this assay, human plasma samples were spiked with an isotope-labeled internal standard solution. Plasma samples were extracted by solid phase extraction (SPE). The analyte PPX was separated from DEX using a chiral HPLC method. An LC-MS/MS system consisting of a Shimadzu LC20-ADXR Prominence UFLC and AB Sciex triple quadrupole mass spectrometer was used. Multiple-reaction monitoring (MRM) and electrospray positive ionization were used for analyte detection. For the qualified assay, the dynamic range of PPX was 0.150–1.00 ng/mL with the lower limit of quantitation at 0.150 ng/mL in the presence of up to 1000 ng/mL of DEX. The minimal pharmacologically active PPX concentration is expected to be greater than 0.300 ng/mL (1). Intra- and inter-day precisions and accuracies were within 80–120% of nominal values for both standards and quality control samples.

Results: A total of 16 samples were tested to determine the presence of pharmacologically significant PPX. PPX was not detected in any of the samples tested (<0.300 ng/mL in the presence of up to 1860 ng/mL of DEX).

Discussion and conclusions: PPX was not detected in humans at pharmacologically significant levels in human plasma after administration of dexpramipexole at doses up to 600 mg/day. Therefore, chiral conversion of DEX to PPX, if it occurs at all, occurs very rarely and does not lead to pharmacologically active PPX in vivo.

Reference

1. Wright CE, Sisson TL, Ichhpurani AK *et al.* J Clin Pharmacol 1997;37:520–525.

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P18 A MULTICENTER, OPEN-LABEL, SINGLE-DOSE, PHARMACOKINETIC AND SAFETY STUDY OF DEXPRAMIPEXOLE IN HEALTHY SUBJECTS AND SUBJECTS WITH RENAL IMPAIRMENT

HE P[1], FARWELL W[1], MARBURY T[2], RIES D[3], STECHER S[1], DONG Y[1], KERR D[1]

[1]*Biogen Idec, Weston, MA, USA,* [2]*Orlando Clinical Research Center, Orlando, FL, USA,* [3]*Twin Cities Clinical Research Center, Minneapolis, MN, USA*

Email address for correspondence: ping.he@biogenidec.com

Keywords: dexpramipexole, pharmacokinetics, renal impairment

Background: Dexpramipexole is currently being investigated for the treatment of amyotrophic lateral sclerosis (ALS). Dexpramipexole has been shown to be primarily renally eliminated and exhibits linear pharmacokinetics across a wide dose range (1).

Objectives: The main objective of this study was to evaluate the pharmacokinetics of a single oral dose of dexpramipexole in healthy subjects and in those with varying degrees of renal impairment. Secondary objectives included a) establishment of a relationship between estimated glomerular filtration rate (eGFR) and dexpramipexole pharmacokinetics and b) evaluation of the safety and tolerability of dexpramipexole in subjects over a range of renal function.

Methods: This was a Phase I, multicenter, open-label, single-dose study. Adult subjects were recruited following an initial screening visit at which a screening eGFR was calculated based on the Modification of Diet in Renal Disease (MDRD) equation. Subjects were grouped by renal function (mild (eGFR 50–79 mL/min/1.73m^2), moderate (eGFR 30–49 mL/min/1.73m^2), severe (eGFR $<$ 30 mL/min/1.73m^2), end-stage renal disease (ESRD, on hemodialysis for at least 3 months)). Twelve healthy volunteers (eGFR $\geq$ 80mL/min/1.73m^2) were matched by age ($\pm$ 10 years) and gender to subjects with renal dysfunction. Each subject received a single dose of dexpramipexole on Day 1; subjects with mild (n = 6) or moderate (n = 6) renal impairment and matched healthy volunteers (n = 8) received 150 mg and subjects with severe renal impairment (n = 6) or with ESRD (n = 6) and matched healthy volunteers (n = 4) received 75 mg (administered day after dialysis for subjects on hemodialysis). In order to identify any unexpected safety or tolerability issues, subjects (referred to as Sentinel) were selected for each renal function cohort (n = 2 for mild and moderate renal impairment and n = 1 for severe and ESRD). Sentinel subjects then progressed through the entire study and had results analyzed prior to dosing the remaining subjects. Urine and blood samples were collected before dosing and for 72 hours post-dosing to determine dexpramipexole concentration. Additional blood samples were collected up to 144 hours in patients with severe renal impairment and ESRD. Laboratory tests, electrocardiograms, vital signs, and adverse event reports were collected to determine safety. Assessments were done for all patients before dosing and on Days 1–4 and Day 7. Patients with ESRD were also assessed on Days 5 and 6.

Results: 36 subjects completed the study as planned. The data are currently under evaluation.

Discussion and conclusions: Pharmacokinetic parameters and safety and tolerability of dexpramipexole in healthy subjects and in subjects with mild, moderate, severe, or ESRD will be reported.

Reference

1. Bozik ME, Mather JL, Kramer WB *et al* J Clin Pharmacol 2011;51:1177–85.

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P19 A RANDOMIZED, BLINDED, PLACEBO-CONTROLLED ASCENDING DOSE STUDY OF THE SAFETY AND PHARMACOKINETICS OF DEXPRAMIPEXOLE IN HEALTHY VOLUNTEERS

FARWELL W[1], HE P[1], LEESE P[2], STECHER S[1], BHARGAVA P[1], PURDY S[1], KERR D[1]

[1]*Biogen Idec, Cambridge, MA, USA,* [2]*Quintiles, Overland Park, KA, USA*

Email address for correspondence: wildon.farwell@biogenidec.com

Keywords: dexpramipexole, pharmacokinetics, safety

Background: Dexpramipexole, a synthetic amino-tetrahydrobenzothiazole, is being evaluated for the treatment of amyotrophic lateral sclerosis (ALS).

Objectives: The primary objective of this study was to determine the safety and tolerability of oral dexpramipexole in doses up to 600 mg as a single dose or 300 mg twice daily for 3.5 days in healthy volunteers. The secondary objective was to evaluate the pharmacokinetics of these doses.

Methods: This Phase 1, single-center, blinded, randomized, placebo-controlled, ascending-dose study was conducted in two parts using 5 cohorts (each cohort was composed of nine healthy adults including at least four females). Randomization within each cohort was 7: 2 dexpramipexole to placebo. Part A (3 cohorts) was a single-ascending dose study (SAD) and Part B (2 cohorts) was a multiple-ascending dose study (MAD). A screening visit to determine study eligibility was conducted in the 28 days prior to dosing. In Part A, each subject received a single oral dose of dexpramipexole (300, 450, or 600 mg) or placebo on Day 1. Serial blood and urine samples were collected prior to dosing and for 72 hours after dosing to determine dexpramipexole concentration. Safety was assessed for all subjects (adverse events, vital signs, clinical laboratory evaluations, electrocardiograms, physical examinations) before dosing, on Days 1–4 and on Day 8 ($\pm$ 1 day). In Part B, subjects received twice daily doses of dexpramipexole (225 or 300 mg) or placebo on Days 1 to 3 and a single dose on the morning of Day 4. Serial blood and urine sampling was done prior to dosing, for up to 12 hours following the initial dose, and for 72 hours following the last dose. Safety assessments were conducted for all subjects before dosing, on Days 1–7, and on Day 11 ($\pm$ 1 day).

Results: A total of 45 subjects completed the study. The data are currently under evaluation.

Discussion and conclusions: Pharmacokinetic parameters following single and multiple doses and safety of up to 600 mg dexpramipexole in healthy volunteers will be reported.

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P20 A SINGLE AND MULTIPLE DOSE, OPEN-LABEL STUDY OF THE PHARMACOKINETICS, SAFETY, AND TOLERABILITY OF DEXPRAMIPEXOLE IN HEALTHY JAPANESE AND CAUCASIAN SUBJECTS

HE P[1], FARWELL W[1], WILBRAHAM D[2], STECHER S[1], BHARGAVA P[1], PURDY S[1]

[1]*Biogen Idec, Cambridge, MA, USA,* [2]*Quintiles, London, UK*

Email address for correspondence: ping.he@biogenidec.com

Keywords: dexpramipexole, pharmacokinetics, Japanese

Background: Dexpramipexole is a potential first-in-class mitochondrial modulator being developed for the treatment of amyotrophic lateral sclerosis (ALS). An ongoing Phase 3 study (EMPOWER) is evaluating the use of dexpramipexole 150 mg twice daily for ALS. Dexpramipexole pharmacokinetics in the Japanese population has not been investigated.

Objectives: The primary objective of this study was to evaluate the pharmacokinetics of single and multiple doses of dexpramipexole in healthy Japanese and Caucasian subjects. A secondary objective was evaluation of the safety and tolerability of single and multiple doses of dexpramipexole in these subjects.

Methods: This study was conducted in 2 parts; for each part, a screening visit was done within 28 days prior to dosing. Subjects of Japanese and Caucasian descent were enrolled in a 1: 1 ratio, matched by gender, age ($\pm$ 10 years), and body mass index (BMI, $\pm$ 20%, if possible) in each study part. Subjects in Part A (n = 28) went through three different treatment periods sequentially: single doses of dexpramipexole 75 mg (Treatment 1) and 150 mg (Treatment 2), then 5 doses of 150 mg twice daily (Treatment 3). There was a wash-out period between treatments. Subjects in Part B (n = 28) received dexpramipexole 300 mg every 12 hours for 5 doses. Blood and urine samples were collected pre-dose and intensively post-dose. Safety assessments (adverse events, laboratory tests, vital signs, 12-lead electrocardiogram, physical examination) were conducted during the treatment days, with a final assessment 6 days after last dosing ($\pm$ 2 days).

Results: The time-plasma concentration profile of dexpramipexole was superimposed between Japanese and Caucasian subjects. At the clinical dose of interest (150 mg twice daily), the geometric mean ratio (C_{max}, AUC) of dexpramipexole exposure between Japanese and Caucasian subjects was close to one (90% confidence interval: 0.80, 1.25) after single dose administration and at steady-state. Renal clearance of dexpramipexole was similar in the two ethnic groups; urinary excretion is the primary elimination pathway for dexpramipexole. Dexpramipexole was generally safe and well tolerated in all participants at doses up to 150 mg twice daily. The pharmacokinetic and safety data at 300 mg twice daily (Part B) are currently under evaluation.

Discussion and conclusions: Pharmacokinetic parameters following single and multiple doses and safety and tolerability of dexpramipexole in healthy Japanese and Caucasian subjects will be reported.

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P21 RECRUITMENT FOR CLINICAL TRIALS INVOLVING FAMILIAL ALS

ALLRED P[1], ANDRES P[2], MAHONEY K[2], JACKSON K[2], CUDKOWICZ M[2], MILLER T[1]

[1]*Washington University, St. Louis, MO, USA,* [2]*Massachusetts General Hospital, Boston, MA, USA*

Email address for correspondence: millert@neuro.wustl.edu

Keywords: SOD1, recruitment, clinical trial

Background: Therapies are being developed for SOD1-familial ALS, which represents 1–2% of ALS. Clinical trials involving this relatively rare patient group requires a focus on recruitment of subjects.

Objectives: To assess recruitment strategies used to recruit 32 SOD1 positive familial ALS subjects for Phase I clinical trial involving the antisense oligonucleotide drug Isis-SOD1Rx.

Methods: Recruitment efforts at the national level included 1) Email alerts sent by non-profit organization to distribute information directly to healthcare professionals 2) Contact information was provided for the coordination center for patient inquires. Appropriate patient inquires were then directed to their closest clinical trial site. 3) Athena Diagnostics, a commercial genetic testing company for SOD1, sent a follow-up letter to the ordering physician or clinic with information about the clinical trial. 4) Travel expenses were reimbursed 5) A patient information webinar was presented. 6) The study sponsor implemented a SOD1 genetic testing program for those with a dominant family history of ALS. 7) Trial subjects were allowed to enroll in more than one cohort. 8) Trial sites focused local efforts towards reviewing their current patient populations for eligible subjects, posting information on institutional websites, and speaking at local patient support groups.

Results: Recruitment and enrollment for the first cohort of 8 subjects took over 10 months to complete. Recruitment and enrollment for cohort 2 and cohort 3 took 3 months and recruitment and enrollment for cohort 4 was completed in 2 months. A total of 21 subjects were enrolled as the protocol allowed for subjects to participate in more than one cohort. There were only three screen failures. Total recruitment and enrollment for this trial was completed in 24 months.

Conclusions: Recruitment for this clinical trial was slow initially. Barriers to recruitment and enrollment included initial lack of genetic testing by the trial, the rural location of the patient population, lack of information about available trials for the specific patient population and some difficulty in refining clinical trial sites for enrollment efficiency. Recruitment for later cohorts was faster. As new therapeutic efforts target specific genetic mutations, one area of clinical trial development and strategy should be to develop mechanisms to cultivate and maintain relationships with families affected by rare genetic disorders to enhance and improve clinical trial enrollment. These mechanisms may include involvement in natural history studies, pre-symptomatic familial studies, and enrollment in genetic and/or biomarker databases.

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P22 PROSPECTIVE ASSESSMENT OF CLINICAL TRIAL CHARACTERISTICS THAT MAY AFFECT EFFICIENCIES OF AMYOTROPHIC LATERAL SCLEROSIS (ALS) CLINICAL TRIALS AND PARTICIPATION BY ALS PATIENTS

LARY C, NEMETH J, FOSTER J, ROUSE R, RUSSO PC, BRAVVER EK, DESAI UG, SANJAK MS, BROOKS BR

Carolinas Medical Center, Department of Neurology, Carolinas Neuromuscular/ALS-MDA Center, University of North Carolina School of Medicine, Charlotte Campus, Charlotte NC, USA

Email address for correspondence: cynthia.lary@carolinashealthcare.org

Keywords: benchmarking, clinical trial recruitment, clinical trial efficiency

Background: At each clinic visit, ALS patients anxiously request opportunities to participate in clinical studies and therapeutic trials. Despite this interest from patients, only a small number are involved in clinical research and attention to the characteristics of such patients identify that they constitute a group of patients with better disease trajectories. To involve ALS patients in such endeavors requires attention by clinicians and clinical researchers to improving time and financial efficiencies that will allow greater patient participation in research embedded within clinical encounters. There are a number of challenges in store for entering more patients in clinical trials.

Objective: Identify characteristics of ALS and neuromuscular disease clinical trials that prevent efficiencies allowing increased numbers of patients to safely participate in more clinical trials.

Methods: Prospective review of clinical research and clinical trial protocols at Carolinas Medical Center Department of Neurology Research Division bi-weekly meeting including MDs and PhD principal investigators, RN coordinators and non-RN study coordinators including subject recruitment/enrollment, study procedures, subject scheduling, adverse events. ALS studies (12) consisted of three intravenous, one enteral feeding, six oral/enteral feeding and two physical interventions. Neuromuscular studies (6) consisted of one intravenous, two oral/enteral feeding interventions and three natural history collections. A center-based biorepository clinical study collected serum/plasma, PBMC, CSF, tissues samples across ALS, neuromuscular and other neurological diseases. Study difficulties and inefficiencies were defined and tabulated prospectively.

Results: Review identified 1) disparities in inclusion/exclusion criteria, 2) no common data elements, 3) different clinical procedures, 4) redundancy in clinimetrics, 5) differences in definition of adverse events (including or not including disease progression), 6) different allowed comorbidities, 7) differences in drug delivery regimen and involvement of investigational pharmacy services, 8) different types of strength measure, 9) inability to perform study procedures and 10) loss of caregiver in advanced patients. Ongoing budget disparities for patient remuneration for participation and non-standard additional costs to finish a clinical trial were additional financial issues that precluded enrolling more patients in clinical trials.

Conclusions: Ten challenges with respect to entering more patients with ALS into clinical trials were identified. Three of these will be addressed by the National Institutes of Neurological Diseases and Stroke Common Data Elements initiative if it becomes the standard of practice. Participation by patients with advanced ALS and the impact of participation on the caregiver has not been adequately addressed in clinical trials to date. As patients with ALS extend their disease trajectory based on standard of care treatment and interventions, then longer clinical trials and more patients will be required.

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P23 COMPARING SURVIVAL WITH AND WITHOUT DIAPHRAGM PACING (DPS)

KATZ J, MILLER RG, MOORE D

California Pacific Medical Center, San Francisco, CA, USA

Email address for correspondence: katzjs@cpmcri.org

Keywords: respiratory, diaphragm pacer, outcomes

Background: With FDA approval, a number of ALS patients will undergo diaphragm pacing (DPS), with the hope of improving survival in coming years. Support for the procedure is based on a comparison to a control group receiving standard respiratory care, but there has still been no clinical trial comparing survival with versus without DPS.

Objectives: To estimate survival outcomes and understand potential biases among patients that underwent DPS. This was done by comparing them to cohorts obtained from two separate studies; one compared early versus standard NPPV (Lechtzin et al.) and a second with the addition of minocycline.

Methods: We measured survival time starting from initiation of NPPV for each patient and applied Cox proportional hazards models to determine the effects of age, gender, site of onset, FVC, riluzole use, and length of time to NPPV on survival. We adjusted covariates that were significant (at $p < 0.05$) in testing whether survival differed in patients receiving DPS compared with the other cohorts.

Results: Follow-up data were available for 77 patients with DPS and 258 without (190 from the Lechtzin study and 68 placebo from the minocycline trial). We found significant differences among the groups in baseline values for FVC, riluzole usage, age and sex. We found that survival was affected by FVC, age, and time to initiate NPPV following diagnosis. The unadjusted hazard ratio for DPS was 0.37 (95% CI 0.27 to 0.51) and 0.40 (95% CI 0.29 to 0.56) after adjustment for FVC, age and time to initiate NPPV. Each HR is highly significant ($p < 0.001$).

Median survival was 26.4 mos (95% CI 19.6 to 42.5) for DPS, 10.4 mos (95%CI 9.0 to 12.9) for Lechtzin study and 10.1 mos (95% CI 8.3 to 13.6) for minocycline placebo patients.

Discussion: Our findings support the hypothesis that DPS improves survival, even after statistically adjusting for imbalances among the study populations. The risk reduction was estimated to be around 60% and median survival was extended by 16 months. We could not exclude certain biases, however. For example, length-time bias (proactive patients seek earlier treatments thus extending the apparent survival time as measured from treatment initiation) and selection bias (sicker appearing patients are denied surgery and given standard care) are not easily quantitated by these studies. An understanding of these concerns is necessary so that a recommendation for the performance of the surgery is carried forward, while a clinical trial is necessary to rule out other factors.

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P24 PROSPECTIVE STUDY OF RADIOTHERAPY OF SALIVARY GLANDS AS TREATMENT OF SIALORRHEA IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

ASSOULINE A[1], BELGHITH B[2], ABDELNOUR-MALLET M[3], DELANIAN S[1,4], LENGLET T[3], BRUNETEAU G[3], SALACHAS F[3], LE FORESTIER N[3], PRADAT P-F[3]

[1]Service de Radiothérapie, Centre Clinique de la Porte de Saint Cloud, Boulogne-Billancourt, France, [2]Service de Radiothérapie, Institut Salah Azaiez, Tunis, Tunisia, [3]Département des Maladies du Système Nerveux, Groupe Hospitalier Pitié Salpêtrière, APHP, Paris, France, [4]Service de Radiothérapie, Hôpital Saint-Louis, APHP, Paris, France

Email address for correspondence: aviassouline@aol.com

Keywords: radiotherapy, salivary glands, sialorrhea

Background: Sialorrhea is a frequent and disabling symptom in patients with amyotrophic lateral sclerosis (ALS). Medical treatment is often poorly effective and/or not well-tolerated. Radiotherapy of salivary glands can be an interesting therapeutic option.

Objective: Estimate efficiency and tolerance of radiotherapy of salivary glands in patients with ALS.

Methods: Prospective monocentric study in ALS patients with sialorrhea treated by radiotherapy in the Clinique De La Porte De Saint Cloud (Boulogne-Billancourt, France). Preliminary results of 40 patients treated between November 2010 and November 2011 are presented. All patients had conformational radiotherapy. Total dose was 10 Gy in 2 fractions on 3 days in 27 patients and 20 Gy in 4 fractions on 10 days in 13 patients. We used two 6 MV photon opposed beams and radiation volume including both submaxillary glands and the two thirds of both parotid glands. Patients had clinical examination at the end of treatment, 1 month and 3 months later. Efficiency of radiotherapy was evaluated with the 9-grades Sialorrhea Scoring Scale.

Results: 32 complete responses (SSS 1, 2) and 8 partial responses (SSS 3, 4, 5) were observed at the end of the treatment. Follow-up at 1 month and 3 months showed a complete response in 16 and 11 patients, respectively, and a partial response in 16 and five patients. Treatment was well tolerated. Acute toxicity was observed in 15 patients : xerostomia in three patients, taste modification in five patients, pharyngeal pain in four patients and thick saliva in three patients. All side effects were resolved in the days following the end of the treatment. Seven patients treated at dose of 10 Gy relapsed and had a second radiotherapy at the same dose with good results.

Discussion: Radiotherapy allowed a dramatic decrease or resolution of sialorrhea. Inter-individual variability explains the relapse in some patients and the dose of 10 Gy is probably not sufficient to maintain a long response. Treatment is well tolerated and toxicity is low with both doses of 10 and 20 Gy. Side effects are transitory even in patients who have a second radiotherapy.

Conclusion: Radiotherapy of salivary glands in ALS patients with sialorrhea appears as a very interesting therapeutic option. A larger number of patients and a longer follow-up remain necessary to confirm these encouraging preliminary results.

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P25 POSTERIOR PHARYNGEAL AUGMENTATION BY AUTOLOGOUS LIPOINJECTION FOR DYSARTHRIA IN ALS

SCHWEIKERT K[1,3], WEBER M[2], WITTE U[4], STORCK C[5]

[1]REHAB Basel, Basel, Switzerland, [2]Muscular Diseases Unit/ ALS Clinic, St. Gallen, Switzerland, [3]Neuromuscular Center University Hospital, Basel, Switzerland, [4]Department of Speech and Language Pathology University Hospital, Basel, Switzerland, [5]Otorhinolaryngology University Hospital, Basel, Switzerland

Email address for correspondence: schweikert.k@gmx.net

Keywords: pharyngeal augmentation, dysarthria, velopharyngeal incompetence

Background: Dysarthria is a motor disorder of articulation, phonation, and respiration, resulting in unintelligibility of speech. In ALS, progressive paresis of bulbar muscles causes velopharyngeal incompetence (VPI) with advancing communication problems. Guidelines recommend alternative and augmentative communication devices. To reduce hypernasality, a nose peg often is uncomfortable and not well accepted by patients. Furthermore, a palatal lift or augmentation prosthesis can be helpful. However, it might be impossible to adapt or wear the prosthesis because of a hyperactive gag reflex or dysphagia. In other conditions of VPI, various surgical management options exist: augmentation of the posterior pharyngeal wall or the soft palate by autologous lipoinjection, is minimally invasive, and has shown effective, lasting results for restoring normal resonance. Therefore, it could also be applied in ALS patients.

Objectives: To report on two patients with ALS who underwent autologous fat injection of the posterior pharyngeal wall for VPI.

Methods: Patients were examined interprofessionally by experts of ALS clinics (a neurologist, an otorhinolaryngologist, a speech pathologist) pre- and postoperatively. Videoendoscopic evaluation of velar function with videographic documentation was made before and two months after surgical treatment. Under general anesthesia, autologous abdominal fat was aspired, centrifugated, and afterwards injected into the posterior pharyngeal wall. Postoperatively, the patients had to wear a flexible abdominal belt for 5 days.

Results: In 2010 and 2011, posterior pharyngeal augmentation was recommended to two male patients with upper-motoneuron-predominant ALS and marked rhinolalia aperta. As gag reflex was clearly increased, palatal prosthesis was not suggested. At the time of operation, the patients were aged 56 and 54 years and had an ALS-FRS-R of 29/48 and 27/48, respectively. Both patients had only mild dysphagia and were orally fed. The disease was characterized by bulbar- and lower-limb-onset, a duration of 7 and 3 years, respectively. Improvement in articulation was demonstrated in both cases postoperatively, and both patients reported that speaking was less fatiguing. As in the 54-year old patient intelligibility of speech deteriorated again, autologous fat grafting was re-applied 7 months later, resulting in improved intelligibility again. Each operation was well tolerated without any side effects.

Discussion: In the field of plastic surgery, fat grafts have become an important procedure with proven long term survival. However, in ALS autologous fat injection of the posterior pharyngeal wall has not been described up to now. Furthermore, distribution and evolution of symptoms substantially differ in ALS patients. Therefore, the

potential benefits, risks, and costs of lipoinjection for VPI are crucial factors.

Conclusions: Dysarthria in ALS remains a significant problem with limited therapeutic options. Augmentation of the posterior pharyngeal wall by autologous fat grafting was a safe and effective treatment in two ALS patients with VPI, and should be considered in selected patients with ALS.

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P26 THE ROLE OF EXERCISE AND ITS IMPACT ON DISEASE PROGRESSION AND SEVERITY IN ALS

CAVANAUGH Z[1,2], YANOSY J[2], KOCZON-JAREMKO B[2], WHITAKER C[1,2], FELICE K[1,2], ANDREWS J[1,2]

[1]*University of Connecticut, Farmington, CT, USA,* [2]*Hospital for Special Care, New Britain, CT, USA*

Email address for correspondence: jandrews@hfsc.org

Keywords: human, exercise, survival

Background: Controversy exists over the possible benefits of physical therapy and exercise as a therapeutic tool to slow disease progression and improve quality of life in ALS. Some studies suggest increased functional ability and reduced disease progression with exercise while others suggest that vigorous physical activity can aggravate excitotoxicity, oxidative stress and increase calcium loads causing selective degeneration of vulnerable motor neurons. Additionally, animal studies suggest that swimming-based therapy may be beneficial (1); however, it is unclear if a specific exercise is more beneficial in ALS patients.

Objectives: To evaluate the impact of exercise in a large cohort of ALS patients with attention to different types of exercise.

Methods: Information from consecutive patients initially evaluated at Hospital for Special Care's (HSC) ALS Clinic between 2007 and 2010 was collected retrospectively. Patients were divided into two groups: those who reported any exercise beyond activities of daily living and those who did not do any additional exercise. The exercise group was further subdivided into types of exercise performed: aerobic, aqua therapy, resistance, and other exercise. Survival, forced vital capacity(FVC) and ALS functional rating scores(ALSFRS) were collected as endpoint measures. Analysis of the data included single factor ANOVA of survival between different types of exercise with confidence intervals. Comparison of means was done with a two sample, t-test and comparison of proportions wasperformed using chi-square test.

Results: Of the 234 ALS patients, 56.8% were male, mean age of disease onset was 63.8 + /-12.6 yrs, 29% were on riluzole, mean initial FVC was 83% + /- 27%, and the initial region of onset was: bulbar(27%), cervical(33%), lumbosacral(37%), thoracic(2%), and respiratory(1%). 27%(62/234) reported exercise: 40.3% aqua therapy, 30.7% aerobic, 9.7% resistance, and 19.4% other. A single factor ANOVA analysis showed no difference in ALS disease duration for different modes of exercise ($p = 0.6867$). 95% confidence intervals showed that aqua therapy promised most with average disease duration between 27.9 and 51.6 months compared to no exercise which showed 95% confidence interval of disease duration between 28.2 and 37.3 months. Although there was not sufficient evidence ($p = 0.1339$), a trend was noted for increased survival in ALS patients who did aqua therapy compared to those who did not. Also, a trend of slower rate of decline inALSFRS (-1.08vs.-1.51,$p = 0.0759$) and lung function (-1.88%vs.-3.36%,$p = 0.0895$) was noted in the exercise group vs. no exercise group.

Discussion and conclusions: There was no significant difference in survival between exercise and no exercise groups suggesting no detrimental effect of exercise in ALS. A trend of improved survival with aqua therapy in ALS patients was noted and is consistent with studies done in ALS mouse models where swimming-based training sustained motor function and increased survival(1).

Reference

1. Deforges S, Branchu J, Biondi O, *et al.* J Physiol. 2009; 587(14):3561–72.

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