Amyotrophic Lateral Sclerosis, 2010; 11: 514–519

informa
healthcare

ORIGINAL ARTICLE

A pilot trial of memantine and riluzole in ALS: Correlation to CSF biomarkers

TODD D. LEVINE[1], ROBERT BOWSER[2], NICOLE HANK[1] & DAVID SAPERSTEIN[1]

[1]*PNA Center for Neurologic Research, Phoenix, Arizona, and* [2]*Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA*

Abstract

The objective of this trial was to determine the safety and tolerability of memantine in patients with sporadic ALS and to examine changes in CSF biomarkers during drug therapy. Twenty patients on stable doses of riluzole were enrolled. Patients received memantine, 10 mg b.i.d., for 18 months. Lumbar punctures were performed at baseline, six and twelve months. The ALSFRS was measured at six weeks, 3, 6, 9, 12 and 18 months. Results showed that patients treated with memantine and riluzole had an average rate of decline on the ALSFRS of –0.73 points per month. Patients who progressed faster than –0.5 ALSFRS points per month had an average baseline CSF tau concentration of 574 pg/ml, while those who progressed slower than –0.5 ALSFRS points per month had CSF tau levels that averaged 298 pg/ml ($p = 0.006$). After therapy with memantine, patients had a 27% decline in CSF tau levels ($p = 0.04$) and four patients whose CSF tau dropped to healthy control levels lost only –0.42 ALSFRS points per month. In conclusion, memantine was well tolerated in patients with ALS. Patients receiving memantine and riluzole lost on average –0.73 ALSFRS points per month. Furthermore, levels of CSF tau at baseline could be correlated with how rapidly a patient's disease progressed.

Key words: *Memantine, Biomarkers, Therapy*

Introduction

At present the only FDA approved drug to treat ALS is riluzole, a medication with several modes of action including an inhibitory effect on glutamate release, inactivation of voltage-dependent sodium channels, and an ability to interfere with intracellular events following neurotransmitter binding to excitatory amino acid receptors. However, the benefits of riluzole are marginal, with pivotal studies showing that riluzole prolonged life by only three months without significantly delaying the loss of functional milestones (1). Thus, a more effective therapy is desperately needed for this universally fatal disease.

There are several lines of evidence suggesting that combination therapy of riluzole and memantine could have a beneficial effect in patients with ALS. Both agents are non-competitive NMDA antagonists that may reduce the effects of glutamate mediated excitotoxicity (2), which has been postulated to play a significant role in the pathogenesis of the disease (3,4). Memantine has been shown to protect neurons against NMDA or glutamate induced toxicity in vitro (5). Memantine can inhibit and reverse the abnormal hyperphosphorylation of tau. Tau hyperphosphorylation leads to protein aggregation and sequestration of other cytoskeletal proteins including microtubule- associated protein 1 (MAP-1) and MAP-2. Furthermore, memantine has been shown to block the disassembly of microtubules which follows the hyperphosphorylation of tau (5). Phosphorylated forms of tau and other cytoskeletal proteins are present in pathological neuronal inclusions (6) and elevated levels of some of these proteins have been found in the cerebrospinal fluid (CSF) of ALS patients in some, but not all, studies (7–9). Finally, memantine has been shown to prolong survival in a mutant SOD1 transgenic mouse model of ALS (10).

We performed an open label pilot trial of memantine used in combination with riluzole in 20 patients with sporadic ALS. Patients were followed for

Correspondence: T. D. Levine, PNA Center for Neurologic Research, 5090 N 40th St., Suite 250, Phoenix, Arizona 85018, USA. E-mail : Levine865@aol.com

(Received 2 February 2010; accepted 1 August 2010)

ISSN 1748-2968 print/ISSN 1471-180X online © 2010 Informa Healthcare
DOI: 10.3109/17482968.2010.513052

18 months. Our primary goal was to assess safety and tolerability, but we also assessed effects on clinical measures of progression and CSF biomarkers.

Methods

Patients

Eligible patients. Twenty patients who met the El Escorial criteria for probable or definite ALS were enrolled. Patients were eligible if they were between the ages of 18 and 85 years, had a forced vital capacity greater than 50% of predicted, and were on a stable dose of riluzole for at least 30 days prior to screening. Informed consent was obtained from all individuals in accordance with institutional review board requirements. All patients underwent a lumbar puncture to measure CSF tau levels at baseline; in order to be enrolled baseline tau had to be elevated (defined as greater than 240 ng/l). This value was set based on previous studies (11) and our unpublished findings for CSF tau levels in healthy controls. Women of childbearing potential were eligible if they were using an effective method of birth control and had a negative pregnancy test prior to enrollment. Patients were excluded if they had a history of liver disease, severe renal failure, intolerance to riluzole, or any other comorbid condition that would make completion of the trial unlikely.

Study procedures

At baseline, a medical history was completed and a physical and neurological exam was performed. Eligible patients received baseline assessments including the ALS functional rating scale (ALSFRS), a quality of life form (SF-36), and a forced vital capacity test (FVC). Eligible patients were given a starting dose of memantine at 5 mg/day for one week, followed by 5 mg weekly titrations up to 10 mg b.i.d.

Patients were seen six weeks after drug initiation, then again at months 3, 6, 9,12 and 18. The primary outcomes measurement, ALSFRS, was administered at each visit by the study coordinator. For patients who died during the study, their last recorded ALSFRS score was used to generate a slope of decline prior to death. The SF-36 was repeated every six months. Lumbar punctures were performed at baseline and at months 6 and 12. Adverse events were recorded as they occurred and were specifically assessed at each scheduled visit. Adverse events were characterized as either unrelated, possibly, probably, or definite related to the study medication. Serious adverse events were considered those resulting in hospitalization or death.

Measurement of CSF biomarkers

CSF samples were collected at a similar time of day for all subjects; 6 cc of CSF were collected into low-bind tubes and frozen in a –80°C freezer for storage. The first few drops of CSF obtained were discarded (to reduce potential blood contamination) and the next 6 cc were collected into the collection vials. All samples were coded in order to maintain patient confidentiality and blinding. Coded samples were sent to separate laboratories for ELISA measurements of candidate biomarkers. Tau levels were measured using a commercially available ELISA kit to total tau (Invitrogen, Carlsbad, CA). Phosphorylated neurofilament heavy chain (pNF-H) concentration in CSF was determined using a human pNF-H ELISA Kit (Millipore, Billerica, MA). In addition, matched CSF samples were sent to Athena Diagnostics for measurement of tau levels to allow more consistent comparisons with pre-study levels and as a way of validating measurements in separate laboratories.

ELISA assays were performed in triplicate within each experiment, and all experiments were performed at least twice. Levels of CSF tau and pNF-H at baseline, month 6 and month 12 were compared to historical and healthy control levels at similar ages. To determine if there was a correlation between the levels of CSF tau or pNF-H and disease progression, patient's disease progression (based on points lost on their ALSFRS scores) were analyzed and stratified into two groups – slower progressors (< 0.5 points per month lost) and faster progressors (> 0.5 points per month lost).

Statistics

Levels of CSF markers were compared between groups by the Mann-Whitney *U*-test (MWU). The unpaired *t*-test was also used for comparison between two groups. Pearson's correlation coefficient was used for correlation analysis between laboratories performing tau ELISA measurements. Analyses were performed using the GraphPad Prism 5.0 software. Statistical significance was determined at $p < 0.05$. Standard box and whisker plots were used to illustrate median, maximum, and minimum values.

Results

Study population

Twenty-two patients were screened for enrollment. Twenty patients who had elevated baseline CSF tau levels ($>$ 240 pg/ml) were enrolled (six females and 14 males). All patients tolerated the maximum dosing titration of 10 mg b.i.d. No patients dropped out or lowered their dose of memantine due to adverse events.

At baseline the average FVC was 75%, average time from symptom onset was one year, average baseline ALSFRS score was 27.8 and the mean age was 63 years.

SF-36 quality of life index

The SF-36 did not indicate appreciable differences in any domain in terms of improving quality of life during the course of the study.

Patient survival

Nineteen patients completed more than three months of memantine therapy and this population was analyzed using their last data point to measure the change in ALSFRS. The one early withdrawal was for psychiatric reasons in a patient with a frontotemporal dementia who was institutionalized eight weeks after beginning therapy. Twelve patients completed the entire 18-month study. Three patients withdrew, all of whom received at least nine months of therapy. Reasons for withdrawing included a patient who wished to participate in another study, one patient who had a prolonged hospitalization due to pneumonia and stopped therapy during the hospital stay, and one patient who was withdrawn for non-compliance. Four patients died during the course of the study (one was in the study for two months, one for five months, one for six months, and one for eight months). In all cases, the cause of death was felt to be related to ALS and not memantine therapy. The average baseline ALSFRS score for the patients who died was 21, which was significantly lower than the average for the others, which was 30 ($p = 0.029$).

ALSFRS and CSF tau correlation

The 19 patients who completed more than three months of therapy were all included in an intent-to-treat analysis. The last recorded ALSFRS score was subtracted from their baseline ALSFRS score and was divided by the number of months that the patients were on treatment to determine a monthly change in ALSFRS score. The 19 patients who received at least three months of therapy lost on average –0.73 points per month and averaged 12 months on therapy. Twelve of these 19 ALS patients were followed in the Banner Good Samaritan ALS clinic for at least three months prior to enrolling in the trial. Therefore, we were able to determine a baseline average disease progression for these patients, which was –1.07 ALSFRS points per month (Figure 1). With an average of 14 months of memantine therapy, these same 12 patients lost on average –0.56 points per month. The ALSFRS scores measured in the clinic setting were carried out by nurses trained to perform the ALSFRS. These nurses were the same raters for the early part of the clinical study. However, there was some turnover in personnel mid-way through the study, which could have introduced some inter-rater variability. Five patients have remained on memantine following the conclusion of the study. They have averaged 28 months on memantine and have progressed at only –0.26 points per month. These patients did not differ significantly from the rest of the group clinically at the onset of the trial.

Levels of CSF tau were measured at both the University of Pittsburgh and Athena Diagnostics using identical ELISA kits. These parallel assessments were both performed blinded to the patient's status as well as to each other. There was a strong correlation between tau levels measured by both laboratories. (correlation coefficient of $r^2 = 0.85$, p-value < 0.0001). Statistical analyses were subsequently performed using ELISA measurements from the University of Pittsburgh since that laboratory had values for pNF-H and tau in untreated ALS patients and healthy controls ($n = 7$) (Figure 4). Both specificity and sensitivity were conducted at 100% based on our study criteria and using only normal controls and only ALS patients with predefined abnormal (> 240 pg/ml) tau levels.

Patients who progressed faster than –0.5 ALSFRS points per month had an average baseline CSF tau level of 574 pg/ml compared to 298 pg/ml in those who progressed at less than –0.5 ALSFRS points per month ($p = 0.006$) (Figure 2). This correlation was also observed in baseline levels of pNF-H. Patients who progressed at rates faster than –0.5 points per month on the ALSFRS scale had levels of pNF-H that averaged 524 pg/ml, while patients who progressed at less than –0.5 points per month averaged 174 pg/ml ($p = 0.002$) (Figure 3). Baseline levels of CSF tau or

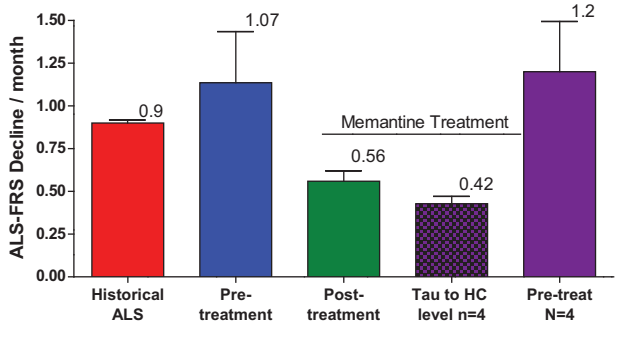

Figure 1. Comparison of the 12 patients who had pre-study ALSFRS scores ($n = 12$). During therapy with memantine, patients' slope of decline of ALSFRS declined to –0.56 points per month. Four patients had CSF tau levels return to normal values and their slope of decline in ALSFRS scores was –0.42. These four patients had pre-treatment progression of –1.2 points per month.

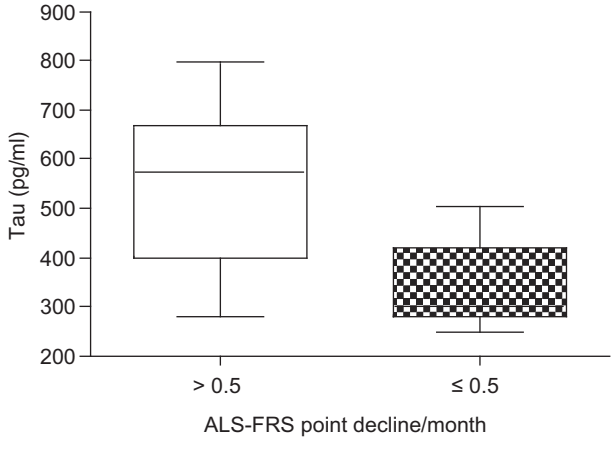

Figure 2. Patients with elevated levels of CSF tau at baseline predicted a more rapid decline based on change in ALSFRS scores ($p = 0.005$).

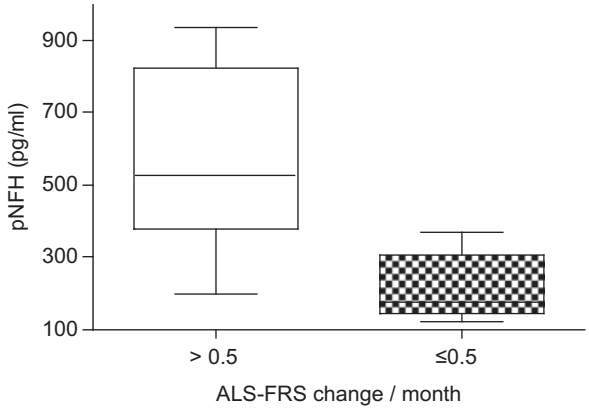

Figure 3. Elevated levels of pNFH in the CSF at baseline were associated with a more rapid progression of disease as evidenced by change in ALSFRS scores per month ($p = 0.002$).

pNF-H showed no significant correlations with patient age, duration of disease, or baseline ALSFRS score. During the course of 12 months of memantine treatment, CSF tau levels showed a significant decline of 27% ($p = 0.04$) (Figure 4). Levels of pNF-H declined as well; however, this change was not statistically significant (25% decline and $p = 0.26$).

During the course of therapy with memantine, 25% ($n = 4$) of the patients corrected their CSF tau levels to levels found in healthy controls (reduced to an average of 230 pg/ml). These patients showed a marked slowing in the progression of their disease, progressing at –0.42 ALSFRS points per month (Figure 1). These same four patients were followed in our ALS clinic before enrollment in the trial and were losing on average –1.2 ALSFRS points per month prior to therapy.

Compliance and safety

Memantine administered at 10 mg b.i.d. was very well tolerated. There was only one adverse event that was considered to be probably related to the study medication. This was a single patient who experienced some nausea during the one-month titration of memantine. This resolved with no specific changes to the study protocol. Three patients required placement of percutaneous gastrostomy tubes during the study because of malnutrition, which were considered serious adverse events but unrelated to the study medication. There were four other hospitalizations that occurred during the course of the study which were labeled as serious adverse events: one patient was admitted to the hospital for a case of epididymitis, one was admitted for chest pain, one admitted for a colon obstruction, and one patient was admitted for fever. These serious adverse events were considered unrelated to the study medication. Three patients died during the course of the study, which was also considered a serious adverse event but felt to be related to the natural progression of their disease and not related to the study medication. A DSMB board met quarterly to assure study compliance as well as safety and tolerability. Compliance was measured through drug accountability. All patients were required to return unused medication at each visit. Drug was accounted for by amount of drug returned subtracted from drug dispensed based on how much drug should have been taken.

Discussion

In this pilot trial of memantine used in combination with riluzole in the treatment of patients with sporadic ALS, there was a strong correlation between CSF levels of tau and phosphorylated heavy neurofilament (pNF-H) and disease progression. Patients who progressed faster than –0.5 ALSFRS points per month had an average CSF tau level of 574 pg/ml, compared to an average level of 298 pg/ml in patients who progressed slower than –0.5 ALSFRS points per month ($p = 0.006$) (Figure 2). After 12 months of treatment with memantine, patients showed a 27% decline in CSF tau; $p = 0.04$). Levels of CSF pNF-H showed a similar correlation to the rate of clinical disease progression (Figure 3) and showed a non-statistical decline following treatment with memantine. Boylan et al. recently published similar results from the serum of patients with ALS, demonstrating that higher levels of pNF-H were associated with a faster rate of progression (16).

As this study did not contain a placebo arm it is difficult to draw conclusions about the significance

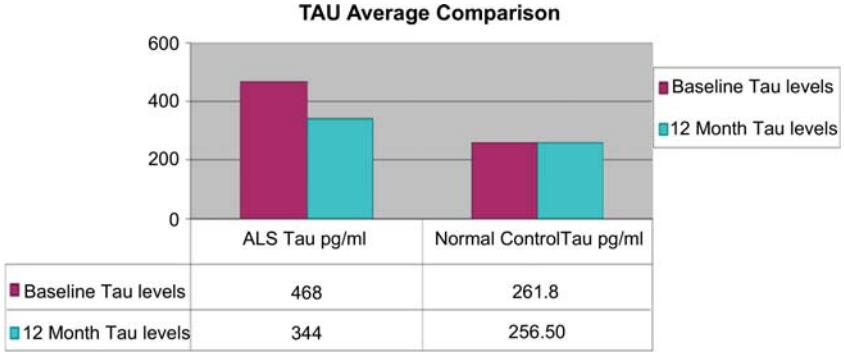

Figure 4. After 12 months of therapy with memantine, CSF tau levels decreased by 27% ($p = 0.04$). In earlier studies normal control patients showed no change in CSF tau levels over 12 months.

of the decline in CSF tau and pNH-H seen during the course of treatment with memantine. Figure 4 illustrates that healthy control subjects do not show changes in CSF tau levels over the course of 12 months. Previous reports have also shown that CSF tau levels do not significantly change over time in patients with a diagnosis of Alzheimer's disease (13). However, as memory impairment worsens and patients progress from mild cognitive impairment to overt Alzheimer's disease there was an observed increase in CSF tau that mirrored the progression of the disease (14). There is also an age-associated increase in the levels of CSF tau, even in ALS patients (15). However, further studies are necessary to evaluate longitudinal CSF tau and pNF-H levels within individual ALS patients.

It is possible that CSF levels of cytoskeletal proteins such as tau and pNF-H correlate to ongoing neuronal injury and death during ALS, which may vary during the course of disease. However, similar longitudinal CSF measurements in ALS patients not receiving memantine treatment are necessary to properly interpret our findings and will be performed in a larger clinical trial design. Our data support continued studies to explore the potential use of candidate biomarker proteins to monitor memantine effectiveness and disease progression in clinical trials.

It is impossible to draw any conclusions about the efficacy of memantine in slowing down the course of the disease given the open label nature of this study. Among the 19 patients who completed more than three months of therapy, the average decline in ALSFRS was –0.73 points per month. This is promising when compared to the average rate of progression of –1.057 ALSFRS points per month (range –0.7 to –1.108) observed in ALS patients in the placebo arms of several completed clinical trials (12). Since this was a single center study we were able to analyze the rate of progression occurring in 12 of the 19 patients prior to the initiation of memantine therapy. These measurements were performed by nurses trained to perform the ALSFRS, but given the fact that the pre-treatment assessments were performed in a clinic setting and the post-treatment assessments were performed in a study setting there could be a significant placebo effect as well as patient or rater biases. Given these limitations, it is still interesting to note that these 12 patients lost, on average, –1.07 ALSFRS points per month prior to enrolling in the study, but during the study their average rate of disease progression slowed to –0.56 ALSFRS points per month.

De Carvalho et al. recently published the results of a placebo controlled trial of 63 patients randomized in a 1:1 fashion to either placebo or 20 mg/day of memantine. This12- month trial showed no effect on progression in ALSFRS or change in electrophysiologic parameters of disease progression (17). Although the numbers from our pilot trial are small, we believe that the potential effects on biomarkers seen in this study support the need for a larger dose-response placebo controlled trial of memantine and riluzole in patients with ALS. Such a trial could validate the changes and correlations in biomarkers seen with memantine therapy, which may suggest that memantine at higher doses could offer neuroprotection via its mechanism as a non-competitive NMDA antagonist. In addition, if the observations seen in this pilot trial are validated in a placebo controlled trial, it would argue that these biomarkers could serve as indicators of response to therapy or disease progression.

Declaration of interest: This study was funded by the Forest Research Institute. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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