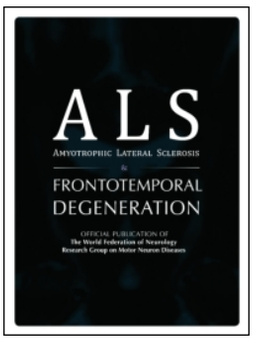

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RESEARCH ARTICLE

Phase I clinical trial of safety of L-serine for ALS patients

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Abstract

We performed a randomized, double-blind phase I clinical trial for six months on the effects of oral L-serine in patients with ALS. The protocol called for enrollment of patients with a diagnosis of probable or definite ALS, age 18–85 years, disease duration of less than three years and forced vital capacity (FVC) $\geq 60\%$. Patients were randomly assigned to four different oral twice-daily dose regimens (0.5, 2.5, 7.5, or 15 g/dose). Blood, urine and CSF samples, ALS Functional Rating Scale-Revised (ALSFRS-R) scores and forced vital capacity (FVC) were obtained throughout the trial. Disease progression was compared with matched historical placebo controls from five previous ALS therapeutic trials. Of 20 patients enrolled, one withdrew before receiving study drug and two withdrew with gastro-intestinal problems. Three patients died during the trial. L-serine was generally well tolerated by the patients and L-serine did not appear to accelerate functional decline of patients as measured by slope of their ALSFRS-R scores. Based on this small study, L-serine appears to be generally safe for patients with ALS.

Keywords: *ALS*, *therapeutic trial*, *L-serine*, *phase I*, *BMAA*

Introduction

Amyotrophic lateral sclerosis/motor neuron disease (ALS/MND) is characterized by progressive degeneration of upper and lower motor neurons, resulting in weakness from muscle denervation and spasticity. Patients typically survive three to five years from diagnosis. Current evidence suggests that ALS results from gene/environment interactions, and that ALS is a syndrome resulting from many causes. Approximately 10% of patients have a familial history of the disease, and more than half of those patients carry a mutation of *SOD1, TDP-43*, *FUS*, *ANG*, *C9orf72* or other known genes (1,2). Environmental causes of ALS, which may play a significant role in sporadic cases, are poorly understood. Exposure to environmental toxins is currently being studied as a possible risk factor for ALS (3–5).

Although L-serine is endogenously produced by astrocytes (6), this amino acid is abundant in human diets; the average American receives 3.5 g per day of L-serine from dietary sources (7). L-serine is currently prescribed, in combination with glycine, for the treatment of two different genetic neurological diseases that result in L-serine deficiency, 3-phosphoglycerate dehydrogenase deficiency and 3-phosphoserine phosphatase deficiency (8) and is under study as a possible treatment for hereditary sensory autonomic neuropathy type 1 (9).

L-serine has been reported from *in vitro* and *in vivo* studies to have neuroprotective effects and may be a potential therapy for Guamanian ALS/Parkinsonism/dementia complex (ALS/PDC) (10–13).

We conducted a phase I clinical trial to test the safety of L-serine for ALS patients. After receiving FDA and IRB approval, 20 ALS patients were enrolled into a double-blind randomized clinical trial of increasing doses of oral L-serine at Phoenix Neurological Associates in Phoenix, Arizona, and

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the Forbes Norris MDA/ALS Research and Treatment Center in San Francisco, California.

Methods

Study design and participants

Twenty patients with probable or definite ALS based on the El Escorial criteria, aged 18–85 years, with an ALS Functional Rating Scale-Revised (ALSFRS-R) score >25 and a forced vital capacity (FVC) of ≥60%, were randomly assigned to four different oral twice-daily L-serine treatment groups in a double-blind six-month trial. The groups received 0.5 g, 2.5 g, 7.5 g, or 15 g twice daily. Exclusion criteria included: symptoms present for more than three years; taking amphetamines or adrenergic agonists such as dopamine; presence of a comorbid condition that could make completion of the trial unlikely; a history of intolerance to L-serine; serum creatinine >2.0 mg/dl; taking any other investigational drug; and, for females of child-bearing age, pregnancy, breast-feeding, or unwillingness to prevent pregnancy during the trial.

We did not recruit a contemporaneous placebo control group of patients with ALS since L-serine is readily available for purchase, and the inclusion of a placebo control group would have inhibited patient enrollment. Instead, we selected 430 ALS patients from the placebo arms of five previous clinical trials who met the same inclusion criteria used for the current phase I L-serine trial. The trial protocols were approved by the Western Institutional Review Board in Olympia, Washington, USA. All patients agreed to participate in the study by written informed consent before inclusion in the trial.

Randomization

After an initial screening visit where patients reviewed and signed the informed consent form, they underwent baseline studies and were randomly assigned to one of the four treatment groups. An unblinded pharmacist provided L-serine in powder form in individual dose packets and retained the dosage code until completion of the trial. Investigators and raters were blinded as to the dose that each patient received.

Procedures

After the initial screening/baseline visit, patients returned monthly for clinical evaluation for six months. At the initial screening/baseline visit, a medical and neurological history was recorded, physical and neurological examinations were conducted, and routine laboratory studies including blood, urine, and cerebrospinal fluid (CSF) were obtained. An ALS Functional Rating Scale-Revised (ALSFRS-R) questionnaire was administered to determine the patient's functional capacity (14). The ALSFRS-R is strongly correlated with functional capacity and survival in ALS patients (14,15). A pulmonary function test was completed using the FVC method with a Puritan Bennett Renaissance II spirometer. Patients were evaluated monthly with the ALSFRS-R scale. The FVC was evaluated at the beginning, middle, and end of the trial. At the end of the trial visit, the patients were re-evaluated with physical and neurological examinations, ALSFRS-R and FVC, and studies of blood, urine and, where possible, CSF.

L-serine powder was prepared in a GMP facility and provided for the study by JoMar Labs, Scotts Valley, California. L-serine powder was dispensed into individual dose packets by an unblinded pharmacist who had no interaction with patients. Doses were taken morning and evening.

Plasma, CSF, and urine samples were analyzed with tandem mass spectroscopy for the presence of the cyanobacterial toxin BMAA using a previously published validated method (11) and for L-serine using a Hitachi Amino Acid Analyzer.

Outcomes

The primary outcome was the safety and tolerability of L-serine at doses up to 15 g twice daily. Secondary outcome measures included survival, and altered decline of functionality as measured by ALSFRS-R scores and FVC.

Statistical analyses

In the safety analysis, we compared the percentage of deaths in this phase I study with that in the historical data in the online PRO-ACT database, and the percentage of withdrawals in our phase I trial with historical data in the phase III clinical trial of minocycline (16). Rate of decline of functionality as measured by ALSFRS-R in L-serine patients was compared with that for a combined dataset of placebo patients from five previous ALS therapeutic trials [NEALS lithium trial ($n = 36$), NEALS Celebrex trial ($n = 81$), NEALS creatine trial ($n = 39$), WALS minocycline trial ($n = 207$), Columbia University qALS trial ($n = 67$)], which had the same enrollment criteria as those for patients in this study ($n = 430$). To assess if L-serine at high dosage might accelerate the progression of ALS we compared the slope of ALSFRS-R decline during the six months of the trial with data from the 430 matched historical placebo controls.

Results

Patient disposition

Twenty patients diagnosed with ALS were enrolled in this randomized double-blind six-month study (see Supplementary Table 1 for patient disposition

after randomization). One patient withdrew after the screening visit and before receiving L-serine because of transport difficulties. One patient withdrew after one month on 0.5 g twice daily because he determined that he was on the low dose and decided to privately purchase L-serine for self-medication at the highest (15 g twice daily) trial dose.

Safety and tolerability

Two patients died during the trial (one after receiving 0.5 g twice daily for four months, one after receiving 7.5 g twice daily for five months). One other patient was enrolled with an FVC of 41% at baseline, which was a protocol violation. This patient had an FVC of 15% at month three and died after five months of 15 g twice daily. In all three fatal cases the site investigator concluded that death was due to progression of ALS and was not related to the study drug.

To determine whether three deaths among 20 ALS patients during six months of follow-up was greater than might be expected, we estimated the probability of surviving six months based on initial FVC value. FVC is an established predictor of survival for ALS patients (17). Kaplan-Meier curves fitted to deciles of initial FVC values from the PRO-ACT database ($n = 3398$) suggest that six-month survival probability is roughly equal to FVC percentage. For example, the six-month survival probability for an ALS patient with an FVC of 41% is 0.41. We had initial FVC measures for 19 of the patients in this study. If we add up their expected survival probabilities, we estimate that 16 of the 19 would be expected to be alive at six months. This is in agreement with the observed number (three) of deaths.

One patient withdrew because of bloating after receiving 15 g twice daily for four months; one patient withdrew after receiving 7.5 g twice daily for one month because of nausea and loss of appetite; and one patient receiving 2.5 g twice daily reported loss of appetite but completed the six-month trial. A total of three withdrawals (15%) in this six-month clinical trial of the safety of L-serine is not unexpected compared with the 11% voluntary withdrawal rate during the four-month lead-in phase of the clinical trial of minocycline (16).

No other adverse events were noted in the remaining study participants, and no significant changes were seen throughout the trial in the routine blood studies, including blood urea and creatinine levels.

The ALS patients in this phase I trial of L-serine were similar in baseline characteristics to the selected 430 historical controls (Table I). When we compared rates of decline of ALSFRS-R in our patients with those in the 430 historical controls, we found a trend of a dose-related decrease in slope (Table II, Figure 1). Rates of decline in FVC did not differ between the two populations.

BMAA and L-serine in blood and CSF

We found that increasing L-serine doses resulted in increasing concentrations of L-serine in blood, urine and CSF (Table III). BMAA was detected in the biosamples of only one patient (a baseline urine sample).

Discussion

In this randomized double-blind phase I trial, we evaluated the safety and tolerability of doses of L-serine up to 15 g twice daily, a dose that approximately doubles the total amino acid intake of the patient. The FDA classified L-serine as GRAS (generally regarded as safe) as a food additive as long as it does not exceed 8.4% of total protein in the diet (CFR Title 21 Section 17.320.18). We found that L-serine appeared to be safe at oral doses ranging from 0.5 g to 15 g twice daily in patients with ALS. We arbitrarily chose six months for the period of the trial to ensure that there was no build-up of toxicity during long-term treatment with L-serine.

The three patients who died had the lowest FVC measurements at baseline (41, 63 and 68%). As noted earlier, the patient with an FVC of 41% was a protocol violation and should not have been included in the study. All three deaths were attributed by the site PI to progression of ALS and unrelated to treatment. Based on FVC and survival data from the PRO-ACT database, three deaths due to ALS could be expected in this cohort during six months of follow-up.

This study has several limitations, including the small sample size, the inherent disease-related heterogeneity of ALS with the resultant risk of cohort effects, and the absence of a concurrent placebo-treated group, necessitating the use of a matched historical placebo control group of patients for

Table I. Baseline comparisons.

Baseline comparisons	Means		Standard deviations		
Measure	Placebo	L-serine	Placebo	L-serine	*p*-value for difference
Initial ALSFRS-R	37.6	39.4	7.38	5.27	0.18
Initial FVC %	90	84	17	19	0.20
Symptom duration (yrs)	1.69	1.36	0.82	0.98	0.16

Table II. ALSFRS-R slope estimates for different statistical models.

Method/Group	Slope (per mo decline)	95% CI	*p*-value for L-serine effect	ALSFRS-R slope reduction
Separate Fits				
Placebo	1.09	(1.01,1.17)		
L-serine	0.76	(0.32,1.20)	0.14	30%
Linear mixed effects models uncorrected				
Placebo	1.06	(0.98,1.14)		
L-serine	0.73	(0.33,1.13)	0.12	31%
Corrected for initial FVC and symptom duration				
Placebo	1.16	(1.07,1.25)	0.044	34%
L-serine	0.76	(0.38,1.41)		
Corrected and with dose[1]				
Linear dose effect[2]	0.06		0.011	
Separate dose effect[3]				
0.5 g	0.16	(-0.60,0.92)	0.68	14%
2.5 g	0.25	(-0.46,0.96)	0.49	22%
7.5 g	0.25	(-0.53,1.03)	0.53	22%
15.0 g	0.99	(0.20,1.78)	0.014	85%

[1]Dose in grams given twice daily.
[2]Estimate is per gram *reduction* in slope of ALSFRS-R.
[3]Estimates are *reductions* in slope at the specified dose.

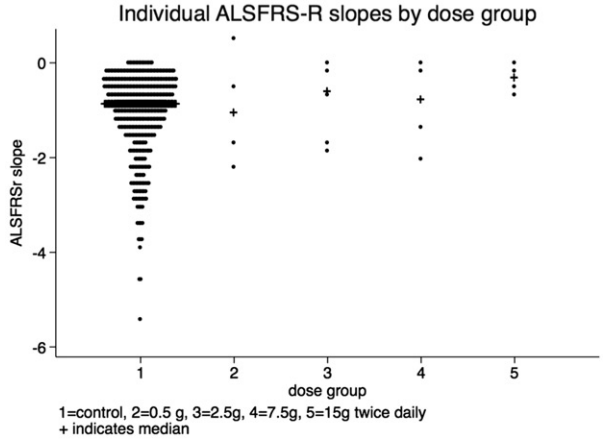

Figure 1. ALSFRS-R uncorrected slope comparison by dose.

Table III. Increasing L-serine dose increases L-serine biospecimen concentrations.

Biospecimen type	*n*	Median L-serine concentrations nmoles/ml in end of study samples 0.5 g BID*	2.5 g BID	7.5 g BID	15.0 g BID
Plasma	12	215	259	435	1464
Urine	12	356	682	1142	2395
CSF	9	36	44	46	137

*BID: twice daily.

comparison. The failure to detect BMAA in blood and CSF samples of ALS patients is consistent with previous literature (18) which indicates that BMAA is primarily found in post mortem brain tissues (19,20).

Based on the findings in this phase I trial, we are planning to proceed to a larger phase II trial involving 66 patients who will receive a dose of L-serine of 15 g twice daily. The dose-response curve of blood L-serine in this phase I study supports the choice of the highest dose for the planned phase II study. Because of gastrointestinal symptoms in three patients taking L-serine out of 20 in this phase I trial, we plan a slow incremental increase in dosage to determine the maximum tolerated dose for each patient in the phase II trial. Since our results indicate that BMAA levels in blood and CSF cannot be used as surrogates for an L-serine effect, we will use the same clinical measures of disease progression used in this phase I study and extend treatment for nine months to explore the possible efficacy of L-serine.

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Declaration of interest: The Institute for Ethnomedicine has applied for a patent for the use of L-serine to treat neurodegenerative illness (US 13/683,821).

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Supplementary material available online