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A phase I, pharmacokinetic, dosage escalation study of creatine monohydrate in subjects with amyotrophic lateral sclerosis

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Abstract

Creatine monohydrate (creatine) has potential neuroprotective properties and is a commonly used supplement in amyotrophic lateral sclerosis (ALS) and other neurodegenerative disorders. Minimum therapeutic and maximum tolerated dosages of creatine are not yet known, nor is it known what systemic plasma concentrations result from specific dosage regimens. The objectives of this study were to establish steady-state plasma pharmacokinetics of creatine at several dosages, and to evaluate the effects of creatine on brain metabolites using proton magnetic resonance spectroscopy (¹H-MRS). Six participants with ALS received creatine at three weekly escalating oral dosages (5, 10, and 15 g b.i.d.). Plasma creatine levels and MR spectra were obtained at baseline and with each dosage increase. Mean pre-dose steady-state creatine plasma concentrations were 20.3, 39.3, and 61.5 ug/ml at 5, 10, and 15 g b.i.d., respectively. Creatine spectra increased by 8% ($p = 0.06$) and glutamate + glutamine signals decreased by 17% ($p = 0.039$) at higher dosages. There were no safety concerns at any of the dosages. In conclusion, creatine plasma concentrations increased in a dose-dependent manner. Creatine appears to cross the blood-brain barrier, and oral administration of 15 g b.i.d. is associated with increased in vivo brain creatine concentrations and decreased glutamate concentrations.

Keywords

Creatine; ALS; imaging; pharmacokinetics; biomarkers

Introduction

Amyotrophic lateral sclerosis (ALS) is the most common type of adult-onset motor neuron disease, clinically characterized by progressive weakness of skeletal muscles and typically fatal within three to five years of disease onset. The cause of selective motor neuron death in ALS is unknown. Many pathogenetic mechanisms have been proposed including mitochondrial dysfunction, glutamate-mediated excitotoxicity, free radical-mediated oxidative cytotoxicity, and apoptotic dysregulation (1). Several potential mechanisms of

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neuronal death in ALS converge at the level of mitochondria. Creatine, an endogenously synthesized amino acid, has been shown to stabilize the mitochondrial transition pore (2), buffer intracellular energy stores (3), stimulate synaptic glutamate uptake (4), and scavenge reactive oxygen species (5). Preclinical evidence suggests that supplementing creatine is neuroprotective in vivo in animal models of neurodegenerative disease states and that the effects are dose dependent (6-9). Human studies with the supplement creatine monohydrate (creatine) at 5 g daily (10,11) and 10 g daily (12) failed to demonstrate efficacy against disease progression in ALS. Experience with creatine in Huntington ' s disease (HD) patients, however, suggests that the compound ' s beneficial effects may not be detected at lower dosages. Creatine at 5 g per day showed no neuroprotective effect in HD patients (13), while 10 g per day for 12 months (14) and 24 months (15) was associated with stabilization of motor and neuropsychiatric function. An unpublished open-label pilot study tested creatine at up to 40 g/day in patients with HD (16). Thus, creatine may be tolerable and neuroprotective at higher dosages than have previously been tested in ALS. The purpose of this study was to establish the plasma pharmacokinetics (PK) of creatine at dosages of 5, 10, and 15g twice daily, and to explore whether creatine crosses the blood-brain barrier by assessing in vivo brain metabolite changes after creatine treatment. This information could facilitate a clinical trial of high dosage creatine in ALS or other disorders.

Materials and methods

Study design

An open-label, single-site, dose escalation study was conducted to assess the plasma pharmacokinetics (PK) and brain metabolite changes of three dosage levels (5, 10, 15 g b.i.d.) of creatine in six participants with ALS. The study was approved by the Massachusetts General Hospital (MGH) Institutional Review Board (IRB).

Study population

Eligible participants were patients with a clinical diagnosis of ALS defined as possible, laboratory-supported probable, probable, or definite ALS by the El Escorial criteria (17). Participants were either not taking riluzole or were on a stable dose of riluzole for ≥ 30 days prior to the baseline visit. Exclusion criteria included known sensitivity to creatine; exposure to creatine within six months of baseline visit; exposure to any investigational agent within 30 days of the baseline visit; presence of any unstable medical illness; significant history of diabetes or renal disease; pregnancy; or a contraindication for MRI studies.

Study procedures

Participants signed an informed consent form prior to screening for the study and they escalated through three different creatine dosage levels of 5 g b.i.d., 10 g b.i.d., and 15 g b.i.d. at days 1, 8 and 15, respectively. Subjects returned for study visits after completing one week of treatment at each dosage level. Study procedures included assessment of eligibility criteria and medical history. Safety procedures were performed at each visit and included review of adverse events, concomitant medications, vital signs, physical examination, electrocardiogram (ECG), and laboratory tests of electrolytes, serum glucose, and liver and kidney function tests. At days 0, 5, 12, and 19, participants underwent MRS with voxels placed within the left frontal cortex.

For assessment of systemic exposure to creatine, blood samples at steady state on days 7, 14, and 21 were drawn prior to and at 0.5, 1, 2, 2.5, and 5 h after administration of dose. Blood samples were centrifuged, and the plasma separated and frozen until the time of assay.

Analysis of plasma samples

Concentrations of creatine and creatinine in plasma were determined by high-performance liquid chromatography (HPLC) with ultraviolet detection. Plasma samples (0.1 ml) were added to analytic tubes together with the internal standard (cimetidine, 10 µg). Calibration standards were prepared in distilled water, to which was added varying known concentrations of creatine (5–200 µg/ml) or creatinine (0.5–20 µg/ml).

Acetone (0.4 ml) was added to study samples and calibration standards, and they were vortex-mixed for 1 min. After centrifugation at $8000 \times g$ for 3 min, the supernatant was separated, evaporated to dryness, reconstituted in 0.1 ml HPLC mobile phase, and centrifuged again at $8000 \times g$ for 1 min.

The HPLC system (Agilent) consisted of autosampler, mobile phase pump, ultraviolet detector, and data processor. The mobile phase consisted of 40% acetonitrile and 60% of a buffer of 100 mM sodium dihydrogen phosphate containing 30 mM sodium lauryl sulfate, with pH adjusted to 3.0 with ortho-phosphoric acid. The flow rate was 0.8 ml/min. The analytic column was 3.9×150 mm containing reverse-phase C-18 Nova-pak (Waters), 4-micron particle size. The ultraviolet detector was set at 200 nanometers.

Under these conditions, approximate retention times were: creatine, 4.4 min; creatinine, 7.3 min; cimetidine, 16.3 min.

Sensitivity limits were 5 µg/ml for creatine and 0.5 µg/ml for creatinine. Variation between replicate samples averaged 1.5% for creatine and 1.45% for creatinine.

Pharmacokinetic analysis

Systemic exposure to creatine and creatinine was evaluated based on the area under the plasma concentration curve (AUC) at steady state for the 0–5-h interval, and the 0–12-h interval, assuming that the concentration at time zero (pre-dose) was equal to the concentration at 12 h. Overall differences among doses in AUC were evaluated using analysis of variance (ANOVA) for repeated measures, with comparison of each dosage level to the other using the Student-Newman-Keuls procedure. The same analysis was carried out on the absolute pre-dose (time zero) plasma levels.

Magnetic resonance spectroscopy (MRS) analysis

All MR experiments were performed in a 3-Tesla MRI system (TimTrio, Siemens Medical Solutions, Malvern, PA) using a 12-channel head coil. Participants were scanned before treatment and at days 5, 12 and 19 after treatment initiation with the exception of patient 6 who missed one scan on day 5. Each scanning session consisted of the following MR sequences:

1. The 'AutoAlign' method of producing scout images was used to improve scan-to-scan reproducibility and to ensure that the slice prescriptions were identical between scan sessions (18).
2. Magnetization Prepared Rapid Gradient Echo (MPRAGE) images were acquired with TI 1100 ms, TR 2530 ms, TE 3 ms, and 1.3 mm slice thickness, $0.9 \text{ mm} \times 0.9 \text{ mm}$ in-plane resolution; and matrix size of 256×256 .
3. Single-voxel 1H MR spectroscopy was performed in the frontal cortex (FC) using a point resolved spectroscopy sequence (PRESS) with WET water suppression (19) using the following parameters: TE = 30 ms, TR = 2000 ms, 128 acquisitions, and a voxel size of $2 \times 2 \times 2 \text{ cm}$.

All spectra were processed offline using the LCModel software package (Provencher, 1993) to determine the quantities of the brain metabolites N-acetylaspartate and N-acetylaspartylglutamate (collectively referred to as NAA), choline-containing compounds (referred to as Cho), creatine + phosphocreatine (referred to as Cr), myo-inositol (MI), and the sum of glutamine and glutamate (referred to as Glx). Metabolite concentrations were estimated using the unsuppressed water signal from the same voxel, which served as the internal standard resulting in institutional units reflecting millimolar concentration and as ratios over choline.

For the serial in vivo MR spectroscopy data, analyses of variance with repeated measures (RM-ANOVA) were employed to detect metabolic changes due to treatment. Due to the missing data point at day 5 post treatment for one participant (P6), RM-ANOVA was performed only for baseline, 10 g b.i.d. and 15 g b.i.d. including all six participants. Subsequently, Dunnett's *t*-tests were used to isolate significant differences between the first scan and the following time-points. All analyses were performed using JMP 7.0 (SAS, Cary, NC).

Results

Seven participants enrolled in the study (Table I). One subject (P7) withdrew while taking 10 g b.i.d. after developing pneumonia that was determined to be unrelated to creatine treatment. All six remaining participants tolerated high creatine dosages and successfully completed the study. The average age was 58 ± 7.8 years and the average disease duration since symptom onset was 34.4 ± 18.6 months.

There were no serious adverse events related to the study drug. One participant had mild elevation in liver enzymes while taking 10 g b.i.d. which normalized at 15 g b.i.d. Three participants developed mild diarrhea. One participant developed diarrhea at 5 g b.i.d. only, another participant at 5 and 10 g b.i.d., and another participant had diarrhea at all dosages. Diarrhea was the only adverse event considered probably related to creatine treatment but was not treatment-limiting. There were no changes from baseline in ECG. Plasma creatinine and glucose levels remained within normal limits for all participants at all visits. Other safety parameters were unremarkable.

Pharmacokinetics results

For creatine, pre-dose, or steady-state trough, plasma concentrations and AUC values differed significantly among the three groups based on ANOVA, and each dosage level differed from the other based on the Student-Newman-Keuls test (Figure 1, Table II). Pre-dose serum concentrations as well as AUC_{0-5} increased proportionally with each dose increment. AUC_{0-12} also increased with increasing dose levels.

Concentrations of creatinine were substantially lower than those of creatine. Creatinine pre-dose concentrations and AUC values tended to increase with higher doses of creatine, but the differences were not statistically significant (Figure 1, Table II).

Magnetic resonance spectroscopy analysis

Creatine concentrations in the frontal cortex revealed a trend towards statistically significant increase (RMANOVA, $p = 0.06$) when comparing spectra at pre-treatment, 10 g b.i.d., and 15 g b.i.d. Creatine increased by 8% comparing pre-treatment and the highest dosage of 15 g b.i.d. (Dunnett's *t* test, $p < 0.05$) (Figures 2-4). Glutamate and glutamine (Glx) concentrations decreased (RM-ANOVA, $p = 0.039$). Glx decreased by 17% between pre-treatment and the highest dosage of 15 g b.i.d. (Dunnett's *t* test, $p < 0.05$). None of the other metabolites such

as N-acetylaspartate (NAA), myo-inositol (MI) and choline (Cho) displayed significant changes after creatine treatment (Figure 4).

Creatine-choline ratio (Cr/Cho) increased with higher creatine dosages (RM-ANOVA, $p < 0.002$), Cr/Cho increased by 15% comparing pretreatment and highest dosage of 15 g b.i.d. (Dunnett 's test, $p < 0.05$) (Figure 5). Glx/Cho decreased by 11% (RM-ANOVA, $p = 0.14$), NAA/Cho increased by 6% (RM-ANOVA, $p = 0.23$) and MI/Cho increased by 4% (RM-ANOVA, $p = 0.26$).

Discussion

ALS is a degenerative disorder of motor neurons that lacks effective treatment and is uniformly fatal. Mitochondrial dysfunction and reduced cellular energy appear to be early and significant features of the disease in animal models and in humans (20-23). Compounds with beneficial effects on mitochondria and cellular energetics are therefore rational therapeutic candidates for ALS. Creatine represents an unusually compelling candidate within this class due to the strength of its preclinical data, emerging clinical evidence in other neurodegenerative diseases, and its known tolerability at a range of dosages (24). The primary purpose of this study was to evaluate the pharmacokinetic properties of escalating high creatine dosages. The secondary purpose was to examine in vivo changes in brain metabolites with increasing doses of creatine.

Our results show a dose-related increase in plasma creatine concentrations with daily use of 5 g b.i.d., 10 g b.i.d., and 15 g b.i.d. MRS results suggest that creatine crosses the blood-brain barrier when given at higher dosages. We also found that brain glutamate-glutamine concentration decreased with increasing brain levels of creatine. The 17% decrease in glutamate concentration was observed after less than three weeks of creatine treatment and is probably biologically meaningful. This finding is of particular interest given the strength of preclinical and clinical evidence for the role of glutamate-mediated injury to motor neurons in ALS.

There is substantial evidence that extracellular accumulation of excitatory amino acids (EAA) such as glutamate and aspartate can induce excitotoxic injury in neurons by accumulating at the synapse and causing repetitive depolarization, cellular edema from sodium influx, and loss of calcium homeostasis. Elevated levels of extracellular glutamate have been found in SOD1 mice and in the cerebrospinal fluid of people with sporadic ALS (1,23,25-28). Riluzole, the only FDA-approved therapy that prolongs survival in ALS, is thought to inhibit glutamate release and attenuate post-synaptic cellular response to glutamate (29,30). Interestingly, creatine has been shown to be a direct energy source for glutamate uptake into the synaptic vesicles and for calcium buffering proteins (4,31).

This study suggests that a possible beneficial effect of oral creatine administration in attenuating cortical glutamate levels may be directly measured as a surrogate study endpoint using MRS. To our knowledge, this is the first in vivo demonstration that brain metabolite levels may serve as a biomarker of drug response for compounds, including creatine, that purport to impact glutamate pathophysiology in human ALS.

Prior clinical trials of creatine in ALS showed no clinical benefit at lower dosages (5 and 10 g/day), whereas higher creatine dosages were neuroprotective in HD (14-16). It is possible that previous ALS clinical trials used sub-therapeutic creatine dosages. This study provides the first investigation of the pharmacokinetics properties of high dosages of creatine and compares these with profiles of brain metabolites. Overall, the results of this study support the notion that 15 g b.i.d. creatine could be tested as a therapeutic dosage in ALS clinical trials. It also stresses the importance of exploring pharmacodynamic biomarkers that may

help determining the optimal dosage before conducting large phase III clinical trials. These data also support MRS as a potential dosage-selection tool when targeting MRS-measurable metabolites and suggest that spectroscopy, along with clinical endpoints, should be further qualified as a potential surrogate marker of drug response in ALS clinical trials targeting glutamate pathways.

Acknowledgments

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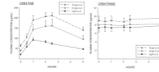


Figure 1.
Mean (\pm SE) plasma concentrations of creatine (left) and creatinine (right) among the six patients at corresponding times.

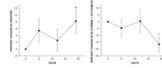


Figure 2. Percent change in Cr and Glx. Percent changes in creatine (Cr) concentrations (left) and glutamate + glutamine (Glx) concentrations (right) observed by in vivo MR spectroscopy in the frontal cortex over the course of creatine monohydrate treatment.

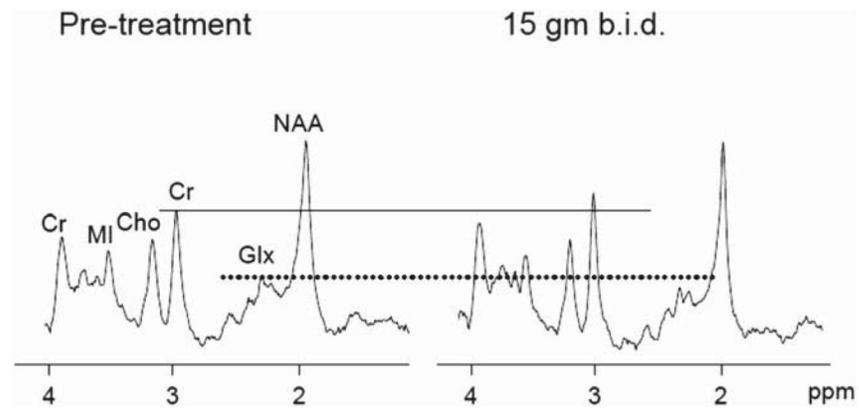


Figure 3. Change in metabolite spectrum. Magnetic resonance spectra of one of the subjects showing increased creatine + phosphocreatine (Cr) and decreased glutamine and glutamate (Glx) concentrations after treatment with creatine 15 g/day.

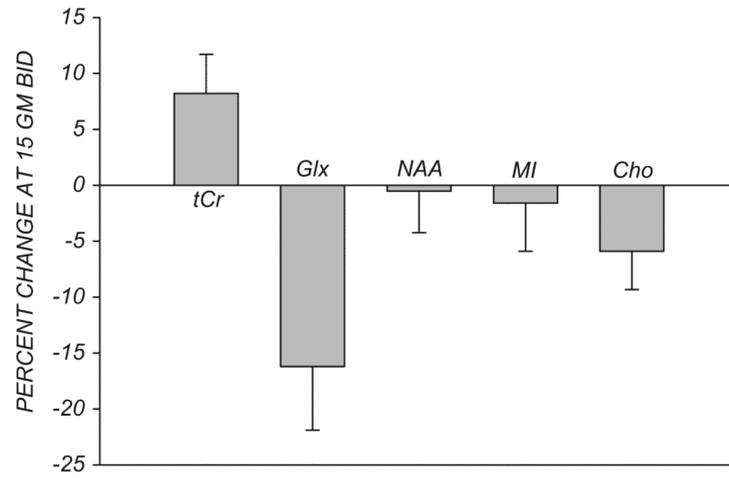


Figure 4. Percent change in metabolites. Percent changes in creatine + phosphocreatine (tCr), glutamate + glutamine concentrations (Glx) N-acetyl aspartate (NAA) myo-inositol (MI) and choline (Cho) observed by in vivo MR spectroscopy between pretreatment and 15 g b.i.d. creatine dosage.

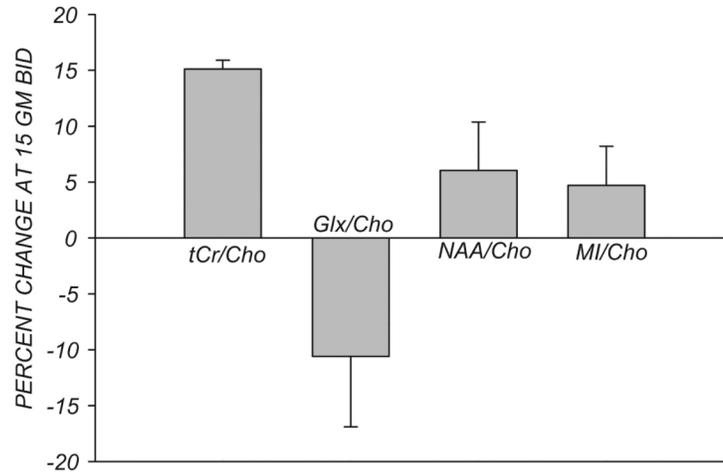


Figure 5. Percent change in metabolites over choline. Percent changes in creatine + phosphocreatine (tCr), glutamate + glutamine concentrations (Glx) N-acetyl aspartate (NAA) and myo-inositol (MI) as ratios over choline (Cho) observed by in vivo MR spectroscopy between baseline and 15 g b.i.d. creatine dosage.

Table 1

Baseline patient characteristics.

	P1	P2	P3	P4	P5	P6	P7
Age (years)	57	59	58	69	43	60	62
Gender	M	M	M	M	M	M	F
Race	W	W	W	W	W	W	W
Onset site	Bulbar	Limb	Limb	Limb	Limb	Limb	Limb
Duration since symptom onset (months)	26	39	21	58	50	4	43
ALS family history	No	No	Yes	No	No	No	No
Riluzole	Yes	Yes	Yes	Yes	No	Yes	Yes

F: female; M: male; P: participant; W: white.

Table II

Analysis of plasma creatine and creatinine concentrations.

	Mean (\pm SE) values for dose			Value of F from ANOVA	Student-Newman-Keuls test		
	5 g b.i.d.	10 g b.i.d.	15 g b.i.d.		5 vs. 10	5 vs. 15	10 vs. 15
Creatine							
Pre-dose concentration ($\mu\text{g/ml}$)	20.3 (\pm 1.3)	39.2 (\pm 4.4)	61.5 (\pm 7.5)	16.5 (p <0.001)	p <0.05	p <0.05	p <0.05
AUC (0–5 h) ($\mu\text{g/ml} \times \text{h}$)	333 (\pm 24)	632 (\pm 110)	857 (\pm 66)	12.1 (p <0.001)	p <0.05	p <0.05	NS
AUC (0–12 h) ($\mu\text{g/ml} \times \text{h}$)	859 (\pm 31)	1101 (\pm 188)	1563 (\pm 134)	13.75 (p <0.001)	p <0.05	p <0.05	p <0.05
Creatinine							
Pre-dose concentration ($\mu\text{g/ml}$)	7.3 (\pm 1.0)	8.0 (\pm 1.0)	9.0 (\pm 1.1)	0.66 (NS)	—	—	—
AUC (0–5 h) ($\mu\text{g/ml} \times \text{h}$)	37.5 (\pm 4.7)	40.4 (\pm 4.9)	45.9 (\pm 5.5)	0.71 (NS)	—	—	—
AUC (0–12 h) ($\mu\text{g/ml} \times \text{h}$)	90.6 (\pm 11.2)	97.0 (\pm 12.2)	111.2 (\pm 14.1)	0.70 (NS)	—	—	—

AUC: area under the curve; h: hour; NS: non-significant; SE: standard error.