

Ketamine Prolongs Survival in Symptomatic SOD1-G93A Mice

John A. Stanford, PhD¹; Matthew Macaluso, MD²; Richard J. Barohn, MD³

¹Department of Molecular & Integrative Physiology, University of Kansas Medical Center, Kansas City, KS 66160, USA

²Department of Psychiatry and Behavioral Neurobiology, University of Alabama at Birmingham, Birmingham, AL 35233, USA

³Department of Neurology, University of Missouri, Columbia, MO 65211, USA

ABSTRACT

Objective. Although riluzole and edaravone are FDA-approved for Amyotrophic Lateral Sclerosis (ALS), these drugs have negligible effect on disease progression and survival. Recent studies reporting neuroprotection from sub-anesthetic doses of ketamine support testing this drug in this rapidly progressing and fatal disease.

Methods. We administered ketamine at 0, 10, and 30 mg/kg to SOD1-G93A mice 5 days/week beginning at 90 days of age. We measured body weight, grip strength, and survival in this model of ALS.

Results. Although ketamine did not influence disease-related loss of body weight, it did delay grip strength declines in the 30 mg/kg group. Ketamine also prolonged survival in the 30 mg/kg group and dose-dependently increased the latency between 20% loss of body weight and death.

Conclusions. These results support further testing of ketamine in preclinical models of ALS to determine optimal dosing. They also support testing in the clinic given the limited efficacy of current ALS treatments and given FDA approval of ketamine for other indications like treatment-resistant depression.

Keywords: *ketamine, survival, SOD1-G93A, neuroprotection*

Introduction

Amyotrophic Lateral Sclerosis (ALS) is a devastating neurodegenerative disease characterized by muscle weakness that rapidly progresses to paralysis due to motor neuron loss in the brain and spinal cord. There is no cure for ALS, and death typically occurs within 5 years of diagnosis (1). There are currently two FDA-approved drug treatments for ALS: riluzole and edaravone. Riluzole is thought to attenuate glutamate-related excitotoxic disease

mechanisms. In addition to blocking sodium channels (2), kainate and NMDA receptors (3), riluzole also facilitates glutamate uptake (4). Edaravone is thought to provide neuroprotection through its antioxidant and free radical scavenging properties (5). Although these drugs are effective against disease mechanisms in ALS, their clinical effects on disease progression and survival are limited (6).

The demonstrated neuroprotective effects of ketamine in other brain disorders (7) makes ketamine an intriguing target of study in neurodegenerative disorders including ALS. These neuroprotective properties of ketamine have been demonstrated in animal models of stroke, traumatic brain injury, and epilepsy (reviewed in (7)). Additional examples of the neuroprotective effects of ketamine have been demonstrated in mouse models of depression, where ketamine treatment resulted in restoration of lost prefrontal cortical spine formations (8). In addition, ketamine prevented neurodegeneration induced by isoflurane anesthesia via anti-apoptotic and antioxidant effects in rats (9). Studies of ketamine for psychiatric disorders in humans date back to the year 2000. The *s*-enantiomer of ketamine was approved by the FDA for treatment-resistant major depression in 2019 and for suicidality associated with depression in 2020.

The mechanism behind ketamine's effects on the brain including its potential neuroprotective effects are complex (10). Unlike riluzole, ketamine likely attenuates NMDA receptor-related glutamate excitotoxicity indirectly. In vitro studies using PC-12 cells reveal that ketamine lowers intracellular D-serine concentrations (11). D-serine is a co-agonist at the NMDA receptor and contributes to NMDA excitotoxicity. Other literature suggests that ketamine treatment has downstream effects on the opioid, aminergic, and cholinergic systems in the brain (12,13).

Given the positive effects of low dose intravenous ketamine on neuropsychiatric disorders and the link between ketamine's mechanism of action and neuroprotection, we performed the first study of sub-anesthetic doses of ketamine in the SOD1-G93A mouse model of ALS.

Materials and Methods

Animals and Dosing. Thirty-six male SOD1-G93A mice were acquired from Jackson Laboratories. Mice were divided into three groups: a ketamine 10 mg/kg group, a ketamine 30 mg/kg group, and a saline vehicle group. After collecting baseline body weight and grip strength data (see below), we administered ketamine or saline vehicle (0.1 ml/kg, ip) 5 days/week beginning at 90 days of age. Drugs were administered following grip strength tests. Procedures were approved by the University of Kansas Medical Center Institutional Animal Care and Use Committee and adhered

to the Guide for the Care and Use of Laboratory Animals.

Grip Strength Testing. Mice were tested for grip strength using an inverted wire screen. Specifically, mice were placed on the screen and then the screen was inverted and held 2 feet above a cushioned surface. The duration that the mice were able to remain on the screen before releasing was recorded across two trials. The mean duration of the two trials was used as the measure of grip strength for each mouse on each day. Mice were tested until they exhibited loss of righting reflex for 30 seconds. At this point they were euthanized. Some mice were found dead in their cage. The day in age for either of these events was recorded as day of death and used for survival analysis (see below).

Data Analysis. Data for body weight and for grip strength were expressed as percentage of pre-drug baseline and analyzed using a 2-way Analysis of Variance (ANOVA) with group assignment (vehicle vs 10 mg/kg vs 30 mg/kg) as the between-subjects variable and testing day (every 7 days) as the within-subjects, repeating variable (Systat 13). Survival analyses were performed using each mouse's day of death (GraphPad Prism). We also compared latencies between the day each mouse lost at least 20% body weight and the day the mouse was euthanized or found dead in its cage using a one-way ANOVA.

Results

Body Weight. Mice in all groups exhibited significant, disease-related weight loss across time ($F=113.765$, $p<0.001$), reaching a nadir of 87% of Day 90 body weight by Day 153 (see Figure 1A).

Body weight loss did not differ between the three

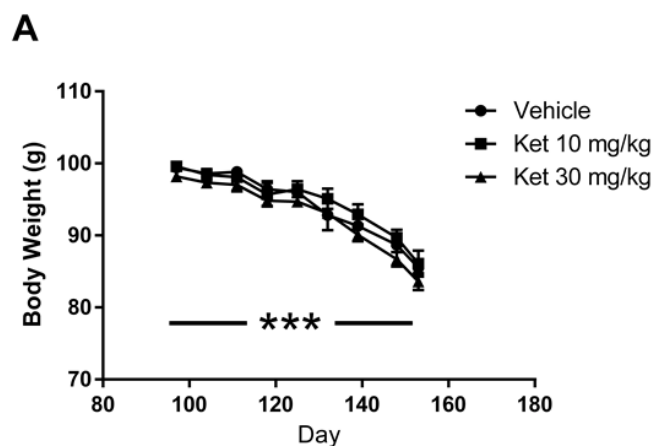


Figure 1A. Body weight as a function ketamine dose and day. Mice in each group exhibited significant loss of body weight (** $p<0.001$). This effect did not differ between dosing groups.

groups.

Grip Strength. Mice in all groups exhibited significant declines in grip strength across time ($F=219.763$, $p<0.001$), reaching -1% of their Day 90 values by Day 153 (see Figure 1B).

The decline in grip strength was less in the 30 mg/kg

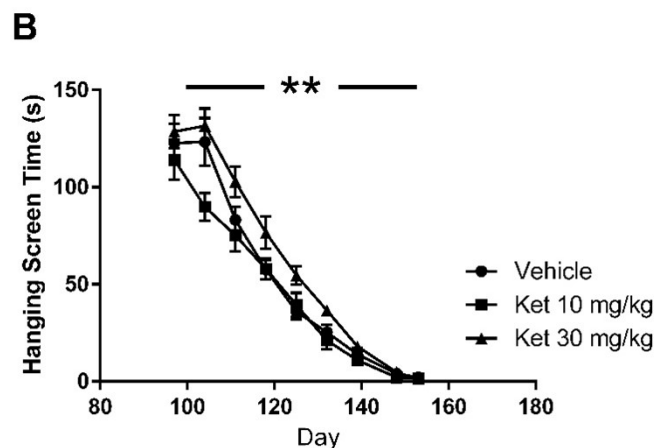


Figure 1B. Grip strength as a function of ketamine dose and day. Although mice in all groups exhibited significant declines in grip strength, the decline was less in the 30 mg/kg ketamine group leading to a significant dose X time interaction (** $p<0.01$).

ketamine group however, leading to a significant main effect for dose ($F=3.697$, $p<0.05$) and a significant dose X time interaction ($F=2.201$, $p<0.01$).

Survival. Survival analysis revealed no significant between-groups differences when all groups were included ($X^2 = 3.578$, $p=0.17$; Figure 2).

A separate analysis that included only the vehicle and 30 mg/kg group, however, revealed significantly longer survival in the ketamine group than the vehicle-treated group ($X^2 = 4.442$, $p<0.05$; data not shown separately, but the curves for vehicle and 30 mg/kg are the same as in

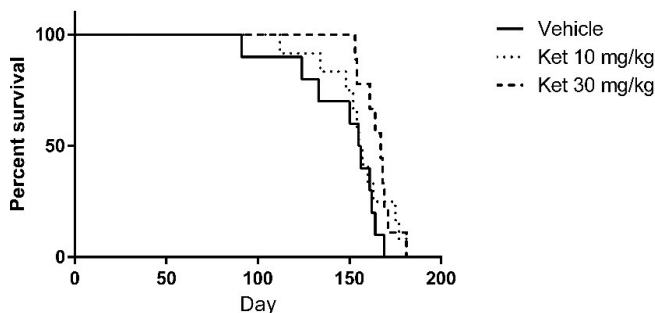


Figure 2. Survival analysis for the three treatment groups. Although survival was greater in the 30 mg/kg ketamine group, the effect did not reach statistical significance when the 10 mg/kg group was included.

Fig. 2). After conducting survival analyses, we measured the latency in days between the day in which each mouse reached 80% of its body weight and the day of death as defined above. Ketamine produced a statistically significant dose-dependent increase in the latency between body weight loss and death ($F=4.642$, $p<0.05$; Fig. 3).

Discussion

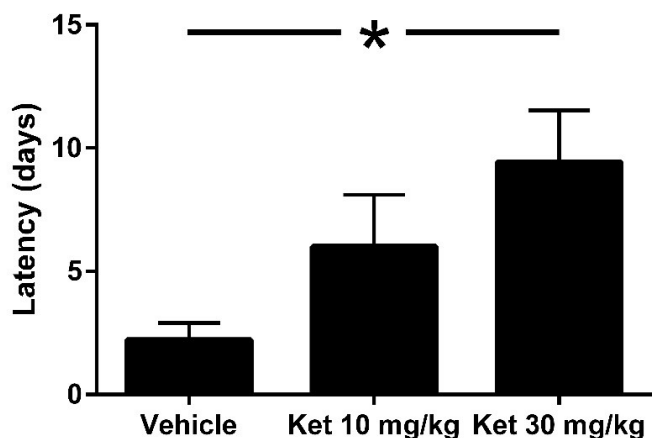


Figure 3. The latency in days between the day each mouse exhibited a $\geq 20\%$ loss of body weight and its day of death. Ketamine produced a significant dose-dependent increase in this measure ($p<0.05$).

We report here that ketamine at sub-anesthetic doses has potential neuroprotective effects in an animal model of ALS. Compared to vehicle-treated controls, mice that were treated 5 days/week with 30 mg/kg ketamine exhibited slower declines in grip strength and prolonged survival. Although ketamine did not attenuate loss of body weight, it significantly increased the latency between 20% loss of body weight and death in a dose-dependent fashion. These data support further testing to determine whether ketamine may help to slow functional decline and improve survival in ALS. Given that ketamine's mechanism of action is similar to riluzole, which is FDA approved to treat ALS, further study of ketamine in animal models of ALS or in human trials may be warranted based on the data presented in this paper.

We tested sub-anesthetic doses of ketamine based on literature supporting the same in treating depression and in promoting neuroprotection, which suggests target modulation of the NMDA receptor at these same doses (14). We chose to dose mice 5 days/week in this study. The fact that neurotoxicity has been reported with repeated ketamine dosing in developing rats and monkeys (15,16) suggests that a more intermittent dosing regimen may result in greater neuroprotection.

It is unclear why mice in the lower dose group exhibited

an earlier decline in grip strength than those in the vehicle and higher dose group. The differential effects may be related to recent reports that ketamine can increase field excitatory postsynaptic potentials (fEPSPs) at doses ≤ 10 mg/kg (17) while decreasing fEPSPs at doses ≥ 30 mg/kg (18). Although speculative, increased excitability may have been detrimental given cortical hyperexcitability at early disease stages in this model of ALS (19,20). This hyperexcitability has been postulated to drive neurodegeneration in motor neurons. The fact that grip strength declines soon followed in the vehicle group but were more delayed in our 30 mg/kg group supports this hypothesis.

The greater survival in the 30 mg/kg group and the dose-dependent increase in latency between loss of body weight and death suggest that, despite muscle wasting, ketamine delayed neuromuscular junction denervation or prolonged neuromuscular function in this model. One shortcoming of the current study was that we did not quantify neuromuscular junction (NMJ) innervation. Future studies in which muscle tissue is harvested from ketamine-treated SOD1-G93A mice at specific disease stages are necessary to determine preservation of NMJ integrity. Our findings suggest that administering a higher dose of ketamine less frequently might provide greater neuroprotective effects in this model. Optimizing the dosing and frequency of administration is a question for further research in this area. In addition, examining target modulation and collecting data on structural and functional changes in the brain before and after treatment would be desirable. Despite these considerations, concurrent human trials should be considered for several reasons: 1) ALS typically causes death within 5 years of diagnosis, 2) the current FDA approved treatments result in no slowing of the disease, and 3) ketamine, a drug which has been available for decades, is now FDA approved in non-anesthetic doses via intra-nasal application for depression (21).

Acknowledgements

This work was supported by the Kansas Intellectual & Developmental Disabilities Research Center (NIH U54 HD 090216).

Corresponding Author

John A. Stanford, PhD
 Department of Molecular & Integrative Physiology
 University of Kansas Medical Center
 3901 Rainbow Blvd., MS 3051
 Kansas City, KS 66160, USA
 jstanford@kumc.edu

References

1. Robberecht W, Philips T. The changing scene of amyotrophic lateral sclerosis. *Nat Rev Neurosci*. 2013;14(4):248-264.
2. Song JH, Huang CS, Nagata K, Yeh JZ, Narahashi T. Differential action of riluzole on tetrodotoxin-sensitive and tetrodotoxin-resistant sodium channels. *J Pharmacol Exp Ther*. 1997;282(2):707-714.
3. Debono MW, Le Guern J, Canton T, Doble A, Pradier L. Inhibition by riluzole of electrophysiological responses mediated by rat kainate and NMDA receptors expressed in *Xenopus* oocytes. *Eur J Pharmacol*. 1993;235(2-3):283-289.
4. Azbill RD, Mu X, Springer JE. Riluzole increases high-affinity glutamate uptake in rat spinal cord synaptosomes. *Brain Res*. 2000;871(2):175-180.
5. Watanabe K, Tanaka M, Yuki S, Hirai M, Yamamoto Y. How is edaravone effective against acute ischemic stroke and amyotrophic lateral sclerosis? *J Clin Biochem Nutr*. 2018;62(1):20-38.
6. Miller RG, Mitchell JD, Moore DH. Riluzole for amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND). *Cochrane Database Syst Rev*. 2012(3):CD001447.
7. Bell JD. In *Vogue: Ketamine for Neuroprotection in Acute Neurologic Injury*. *Anesth Analg*. 2017;124(4):1237-1243.
8. Moda-Sava RN, Murdock MH, Parekh PK, Fectho RN, Huang BS, Huynh TN, Witztum J, Shaver DC, Rosenthal DL, Alway EJ, Lopez K, Meng Y, Nellissen L, Grosenick L, Milner TA, Deisseroth K, Bito H, Kasai H, Liston C. Sustained rescue of prefrontal circuit dysfunction by antidepressant-induced spine formation. *Science*. 2019;364(6436):eaat8078.
9. Wang R, Zhang Z, Kumar M, Xu G, Zhang M. Neuroprotective potential of ketamine prevents developing brain structure impairment and alteration of neurocognitive function induced via isoflurane through the PI3K/AKT/GSK-3 pathway. *Drug Des Devel Ther*. 2019;13:501-512.
10. Potter DE, Choudhury M. Ketamine: repurposing and redefining a multifaceted drug. *Drug Discov Today*. 2014;19(12):1848-1854.
11. Singh NS, Rutkowska E, Plazinska A, Khadeer M, Moaddel R, Jozwiak K, Bernier M, Wainer IW. Ketamine Metabolites Enantioselectively Decrease Intracellular D-Serine Concentrations in PC-12 Cells. *PLoS One*. 2016;11(4):e0149499.
12. Williams NR, Heifets BD, Blasey C, Sudheimer K, Pannu J, Pankow H, Hawkins J, Birnbaum J, Lyons DM, Rodriguez CI, Schatzberg AF. Attenuation of Antidepressant Effects of Ketamine by Opioid Receptor Antagonism. *Am J Psychiatry*. 2018;175(12):1205-1215.
13. Sleigh J, Harvey M, Voss L, Denny B. Ketamine – More mechanisms of action than just NMDA blockade. *Trends Anaesth Crit Care*. 2014;4(2-3):76-81.
14. Fava M, Freeman MP, Flynn M, Judge H, Hoepfner BB, Cusin C, Ionescu DF, Mathew SJ, Chang LC, Iosifescu DV, Murrough J, DeBattista C, Schatzberg AF, Trivedi MH, Jha MK, Sanacora G, Wilkinson ST, Papakostas GI. Double-blind, placebo-controlled, dose-ranging trial of intravenous ketamine as adjunctive therapy in treatment-resistant depression (TRD). *Mol Psychiatry*. 2020;25(7):1592-1603.
15. Hayashi H, Dikkes P, Soriano SG. Repeated administration of ketamine may lead to neuronal degeneration in the developing rat brain. *Paediatr Anaesth*. 2002;12(9):770-774.
16. Slikker W Jr, Zou X, Hotchkiss CE, Divine RL, Sadvovova N, Twaddle NC, Doerge DR, Scallet AC, Patterson TA, Hanig JP, Paule MG, Wang C. Ketamine-induced neuronal cell death in the perinatal rhesus monkey. *Toxicol Sci*. 2007;98(1):145-158.
17. Aleksandrova LR, Wang YT, Phillips AG. Ketamine and its metabolite, (2R,6R)-HNK, restore hippocampal LTP and long-term spatial memory in the Wistar-Kyoto rat model of depression. *Mol Brain*. 2020;13(1):92.
18. Li Y, Ding R, Ren X, Wen G, Dong Z, Yao H, Tan Y, Yu H, Wang X, Zhan X, Yao J, Lu Y, Zhang G, Wu X. Long-term ketamine administration causes Tau protein phosphorylation and Tau protein-dependent AMPA receptor reduction in the hippocampus of mice. *Toxicol Lett*. 2019;315:107-115.
19. Pieri M, Carunchio I, Curcio L, Mercuri NB, Zona C. Increased persistent sodium current determines cortical hyperexcitability in a genetic model of amyotrophic lateral sclerosis. *Exp Neurol*. 2009;215(2):368-379.
20. Saba L, Viscomi MT, Martini A, Caioli S, Mercuri NB, Guatteo E, Zona C. Modified age-dependent expression of NaV1.6 in an ALS model correlates with motor cortex excitability alterations. *Neurobiol Dis*. 2019;130:104532.
21. US Food and Drug Administration. FDA approves new nasal spray medication for treatment-resistant depression; available only at a certified doctor's office or clinic. <https://www.fda.gov/news-events/press-announcements/fda-approves-new-nasal-spray-medication-treatment-resistant-depression-available-only-certified>. Accessed November 27, 2020.