

## 3,4-diaminopyridine Phosphate in Symptomatic SOD1-G93A Mice

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### ABSTRACT

**Objective:** To study the effect of 3,4-diaminopyridine phosphate (3,4-DAP) on body weight, grip strength, neurological score and survival in symptomatic SOD1-G93A mice.

**Method:** We administered 3,4-diaminopyridine phosphate (3,4-DAP) at 0, 8, and 16 mg/kg to SOD1-G93A mice 5 days/week beginning at 90 days of age. We measured body weight, grip strength, neurological score and survival in this model of ALS.

**Results:** 3,4-DAP had no influence on body weight, grip strength, neurological score or survival in this transgenic mouse model.

**Conclusion:** Our study showed that 3,4-DAP administration had no effects on survival, bodyweight, grip strength and neurological score of mice with SOD1 G93A mutation with intervention starting at 90 days of age. We believe that larger animal studies, longer treatment times and/or earlier in life treatment are required to further investigate the utility of 3,4 DAP in ALS patients.

### Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal and rapidly progressive motor neuron disease characterized by the selective loss of corticospinal neurons within the motor cortex and of a-motor neurons of the spinal cord and brainstem.<sup>1</sup> There is progressive muscle weakness, atrophy, and spasticity, which reflect the degeneration and death of upper and lower motor neurons and muscle denervation.<sup>1</sup> There is some experimental evidence that supports the “dying back hypothesis” that postulates that

ALS begins with the degeneration of the neuromuscular junction (NMJ), which results in axonal degeneration and, finally, motor neuron loss.<sup>1,2</sup> Both pre- and postsynaptic alterations interact with synaptic components of the NMJ and contribute to the progression of ALS.<sup>1,2</sup>

3,4-diaminopyridine (3,4-DAP) is a quaternary ammonium compound, which increases the release of acetylcholine in the neuromuscular synapse by prolonging the activation of calcium influx at the nerve terminal.<sup>3</sup> It selectively blocks potassium channels in nerve membranes owing to an enhanced influx of calcium ions by potassium blockade and the resulting in prolongation of the nerve terminal action potential.<sup>3,4</sup> By this mechanism 3,4-DAP increases impulse-evoked transmitter release from motor nerve terminals.<sup>3,5</sup>

3,4 DAP has shown to improve motor weakness for a short period of time by enhancing peripheral synaptic efficiency and has been used for symptomatic treatment of motor impairment due to multiple sclerosis and Lambert Eaton myasthenia syndrome (LEMS).<sup>5,6</sup> A couple of double blinded studies in the past have noted functional motor status improvement with 3,4 DAP along with rehabilitation in ALS patients.<sup>7,8</sup>

We used 3,4-DAP to observe if increasing neurotransmitter availability in the neuromuscular junction would have any effect on grip strength, body weight, neurological score and survival in symptomatic SOD1-G93A mice.

### Materials and Methods

**Animals and dosing:** Thirty-six male SOD1-G93A mice were acquired from Jackson Laboratories. Firdapse (3,4-DAP) was obtained from Catalyst Pharmaceuticals, Inc. Mice were divided into three groups: a 3,4-DAP 8 mg/kg group, a 3,4-DAP 16 mg/kg group, and a saline vehicle group. After collecting baseline body weight and grip strength data (see below), we administered 3,4-DAP or saline vehicle (10 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

ethanized. 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).

Neurological score: Both hind legs were assessed daily for each mouse from 50 days of age and neurological score was calculated using a scale 0 to 4 that was developed by observation at ALSTDI. Criteria used to assign each score level were:

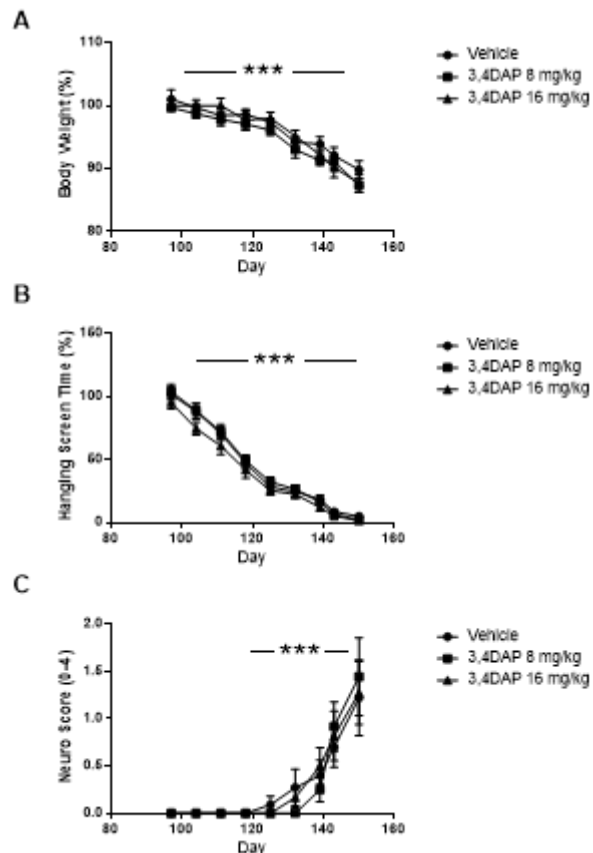
- 0- Full extension of hind legs away from lateral midline when mouse is suspended by its tail, and mouse can hold this for 2 seconds, suspended 2-3 times.
- 1- Collapse or partial collapse of leg extension towards lateral midline (weakness) or trembling of hind legs during tail suspension.
- 2- Toes curl under at least twice during walking of 12 inches, or any part of foot is dragging along cage bottom/table.
- 3- Rigid paralysis or minimal joint movement, foot not being used for forward motion.
- 4- Mouse cannot right itself within 30 seconds from either side.

Data Analysis: Data for body weight, grip strength and neurological score were expressed as percentage of pre-drug baseline and analyzed using a 2-way Analysis of Variance (ANOVA) with group assignment (vehicle vs 8 mg/kg vs 16 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.

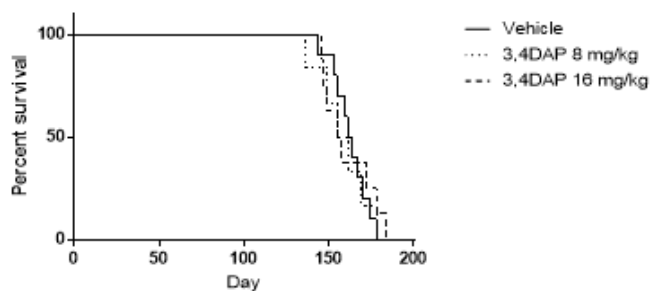
## Results

On analysis of the variables, we found that there was no significant change in the variables throughout the study period in the mice with SOD1 mutation.

Although there was no significant association noted between 3,4-DAP and body weight, mice who were administered 16mg/kg of 3,4 DAP showed slightly higher body weight through the study period as compared to those who were administered the 8mg/kg dose. A similar trend is noted for the grip strength, neuro score and survival as well. These results are depicted in figure 1A, 1B, 1C and figure 2.



**Figure 1.** (A) Body weight, (B) Grip strength (measured by hang time), and (C) Neurological score (0 = normal, 1-3 = progressively abnormal hindlimb behavior during tail suspension, 4 = loss of righting reflex; see Neurological Scoring System at the end of this document). None of these measures were affected by 3,4-DAP. Asterisks indicate significant main effect of Day (disease) on mice in the three groups.



**Figure 2.** Survival was not affected by the administration of 3,4-DAP.

## Discussion

3,4-DAP increases the release of acetylcholine in the neuromuscular synapse by prolonging the activation of voltage gated calcium channels at the nerve.<sup>3,4</sup> By this mechanism, it enhances neuronal excitability and improves neuromuscular and central synaptic transmission.<sup>3,4</sup> It has been successfully used to improve muscle strength and fatigue in Lambert-Eaton myasthenic syndrome and multiple sclerosis patients.<sup>5,6</sup>

NMJ integrity tightly depends on the presynaptic release of acetylcholine and on the clustering of acetylcholine receptors on the muscle plasma membrane to trigger muscle action potentials.<sup>2</sup> Immunofluorescence studies have shown that the density of synaptic vesicles in motor axon terminals from mutant SOD1 mice was significantly reduced compared to the wild-type.<sup>2</sup> In a study by Clark et al,<sup>9</sup> distal axonal and NMJ alterations were noted in muscles of SOD1 G93A mice even before the onset of the clinical symptoms of ALS.<sup>9</sup>

Although there is no current translational application, a couple of double blinded studies in the past have noted functional motor status improvement with 3,4 DAP along with rehabilitation in ALS patients.<sup>7,8</sup> In the recent years there has been discussion regarding how NMJ pathology has relatively received little attention in ALS and it has been proposed that additional research should focus on the potential of preserving NMJs in order to delay or prevent disease progression.<sup>10</sup> The above study was an attempt in that direction.

The conclusion of the above study is that 3,4-DAP administration had no effects on survival, bodyweight, grip strength and neurological score of mice with SOD1 G93A mutation with intervention starting at 90 days of age. We believe that larger animal studies, longer treatment times and/or earlier in life treatment are required to further investigate the utility of 3,4 DAP in ALS patients.

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