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Gonadectomy and dehydroepiandrosterone (DHEA) do not modulate disease progression in the G93A mutant SOD1 rat model of amyotrophic lateral sclerosis

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Abstract

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease characterized the progressive loss of brain and spinal cord motor neurons. Epidemiological studies have shown a higher incidence of ALS in men than women. Interestingly, there are clear gender differences in disease onset and progression in both mouse and rat models of familial ALS over-expressing mutated human superoxide dismutase-1 ($SOD1^{G93A}$). To determine a possible involvement of sex steroids and neurosteroids in ALS, we first asked whether the reduction of serum steroid levels by gonadectomy can modulate disease progression in $SOD1^{G93A}$ rats. Although sexual dimorphism was observed in intact and gonadectomized $SOD1^{G93A}$ rats, there is no significant effect of gonadectomy on disease onset and progression. We next tested whether hormonal treatment using a neurosteroid dehydroepiandrosterone (DHEA) can ameliorate motor neuron loss in both sexes of $SOD1^{G93A}$ rats. DHEA treatment did not alter disease progression or survival in $SOD1^{G93A}$ rats. Our results indicate that gonadal steroids and neurosteroids are not one of the possible modulators for the occurrence or disease progression in a rat model of ALS. Further analysis will be necessary to understand how sexual dimorphism is involved in ALS disease progression.

Keywords

Amyotrophic lateral sclerosis (ALS); $SOD1^{G93A}$ rats; sexual dimorphism; gonadectomy; dehydroepiandrosterone (DHEA)

Introduction

The exact etiology of Amyotrophic lateral sclerosis (ALS) is still unclear, but most epidemiological studies have shown a higher incidence of ALS in men than women (1). Sexual dimorphism in disease onset and progression is also observed in mouse (2;3) and rat (4) models for familial ALS using mutant human superoxide dismutase 1 gene ($SOD1^{G93A}$). Furthermore, it has been demonstrated that ovariectomy led to a significant acceleration of disease progression in $SOD1^{G93A}$ female mice (3;5). These observations suggest an involvement of sex steroids on the occurrence or disease progression of ALS.

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Steroids have pronounced neurotrophic effects on normal neuronal activity during development and in adulthood. Particularly, neurosteroids such as dehydroepiandrosterone (DHEA) are the most abundant steroids in the blood of young adult humans and have neuroprotective properties *in vitro* (6;7) and *in vivo* (8;9). Significant interest has arisen from the hypothesis that declining DHEA concentrations in adults may serve as an indicator of a number of conditions (10).

In the present study we asked whether the alternations of serum steroid levels by gonadectomy or chronic DHEA can modulate disease progression in $SOD1^{G93A}$ rats.

Materials and methods

We prepared a cohort of $SOD1^{G93A}$ rats as described previously (4) in accordance with the guidelines for UW-Madison and NIH standards of animal care. Male (n=8) and female $SOD1^{G93A}$ rats (n=9) were gonadectomized at 60 days of age. Chronic DHEA treatments were performed by using medical grade silastic tubing implants or hormone pellets as described previously (11). $SOD1^{G93A}$ rats (85 days old) received subcutaneous implants into the cervical region with a silicone-tube containing crystalline DHEA (Sigma-Aldrich) or cholesterol (control). At endpoint, trunk blood samples were collected and measured serum steroid concentration by radioimmunoassay.

We performed the routine analyses of locomotor testing using the Basso-Beattie-Bresnahan (BBB) score until endpoint as previously described (4;12). Disease onset was estimated by using the BBB rating score of 16 or lower (4). Statistical analysis of disease onset and survival was performed using the Kaplan-Meier method with log rank test. In Table 1, survival periods of the animals were expressed as means ± SEM and analyzed by two-tailed *t*-test. Differences in BBB score were calculated using two-way ANOVA with Bonferonni *post hoc* test. Differences were considered significant when $P<0.05$.

Results

As we have shown previously (4), survival periods for intact females were significantly longer when compared to males (Fig. 1; $P<0.05$). Gonadectomy did not affect disease onset in either males (Fig. 1A) or females (Fig. 1C) when compared to the non-surgery control $SOD1^{G93A}$ rats. Furthermore, there was no significant difference in survival between gonadectomized $SOD1^{G93A}$ rats and their same-sexual counterparts from onset until death (Figs. 1B and D). The mean time to reach endpoint was 141 days in control males (n=10), 161 days in control females (n=11), 142 days in gonadectomized males (n=8), and 162 days in gonadectomized females (n=10) (Table 1). We next analyzed whether gonadectomy affects to motor function in $SOD1^{G93A}$ males and females. Regardless of the treatment, SOD^{G93A} females showed higher BBB scores when compared to $SOD1^{G93A}$ males ($P<0.05$). However, gonadectomy did not alter progression of motor dysfunction in both SOD^{G93A} males and females (Fig. 2).

Serum DHEA concentrations were significantly higher in DHEA treated animals (6.6 ± 3.1 ng/ml in males, 10.6 ± 4.9 ng/ml in females), compared to the levels in control males (0.4 ± 0.3 ng/ml) and females (1.2 ± 0.5 ng/ml). However, disease onset progressed in a similar fashion between the two groups for each gender (Fig. 3A and 3B). The survival percentage of the rats demonstrates this trend as well (Fig. 3C and 3D). In this cohort, the means ± SEM to reach endpoint were 177 ± 16.7 days in control males (n=11), 190 ± 13.0 days in control females (n=11), 171 ± 16.3 days in DHEA-treated males (n=7), and 186 ± 8.3 days in DHEA-treated females (n=7). The BBB scores of the ALS males and females and those of

both sexes receiving DHEA also support the idea that DHEA does not alter disease progression (data not shown).

Discussion

There is no significant effect of gonadectomy on disease onset or progression in SOD1^{G93A} rats. In SOD1^{G93A} mice, ovariectomy accelerated disease progression in females (3;5). The causes for dissimilarities observed here are uncertain at this point, but the difference of species or the timing of the gonadectomies may be a possible reason. In previous experiments using SOD1^{G93A} female mice, ovariectomy was performed at postnatal day 50 (3;5). While our SOD1^{G93A} rat colony indicates the median to reach disease onset and endpoint is 146 and 184 days (13), SOD1^{G93A} mice in those papers showed 70–80 and 130–140 days as disease onset and endpoint, respectively (3;5). This idea of a specific time window that shows the effects of sex steroids could be further analyzed by doing differently timed gonadectomies in SOD1^{G93A} rats. Furthermore, this is the first study to assess the potential role of castration and testicular androgen in a rodent model of ALS. Free testosterone, which can go through the blood-brain barrier as unbound form and potentially metabolize to estrogen in the nervous system, was significantly decreased in the ALS patients of both sexes (14). Further studies are necessary to elucidate how gonadal steroids are linked to the pathophysiology of ALS using different rodent models of ALS.

DHEA has no effect on ALS disease onset, motor function, or survival in both male and female SOD1^{G93A} rats. In a similar fashion to the gonadectomies, examining the ideal dosage of DHEA along with studying the possibility of a time window to see the effects of this neurosteroid could be necessary. However, these current possibilities would suggest that DHEA, which is currently available as a health supplement in the United States, should be carefully considered as a treatment for ALS.

In conclusion, our results indicate that steroids are not one of the possible modulators for the occurrence or disease progression in a rat model of ALS. Further analysis will be necessary to understand how sexual dimorphism is involved in ALS disease progression.

Acknowledgments

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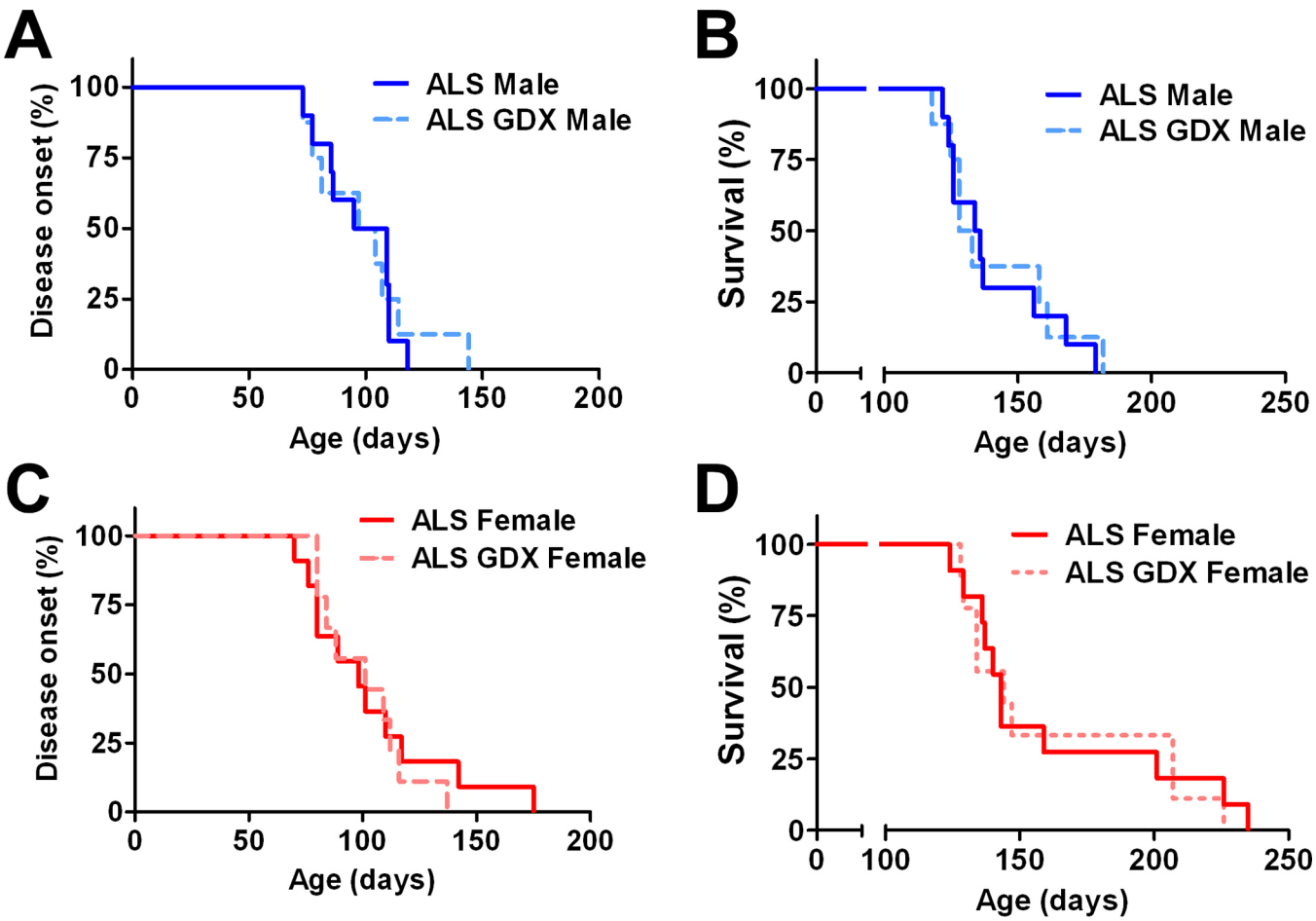

Figure 1. Effects of gonadectomy on disease onset and survival in SOD1^{G93A} rats

Kaplan-Meier curves generated from gonadectomized and intact SOD1^{G93A} rats depicting disease onset and survival. Castration did not affect to disease onset (A) and survival period (B) in SOD1^{G93A} males. Ovariectomized SOD1^{G93A} females showed similar trends in disease onset (C) and survival (D) compared to intact females.

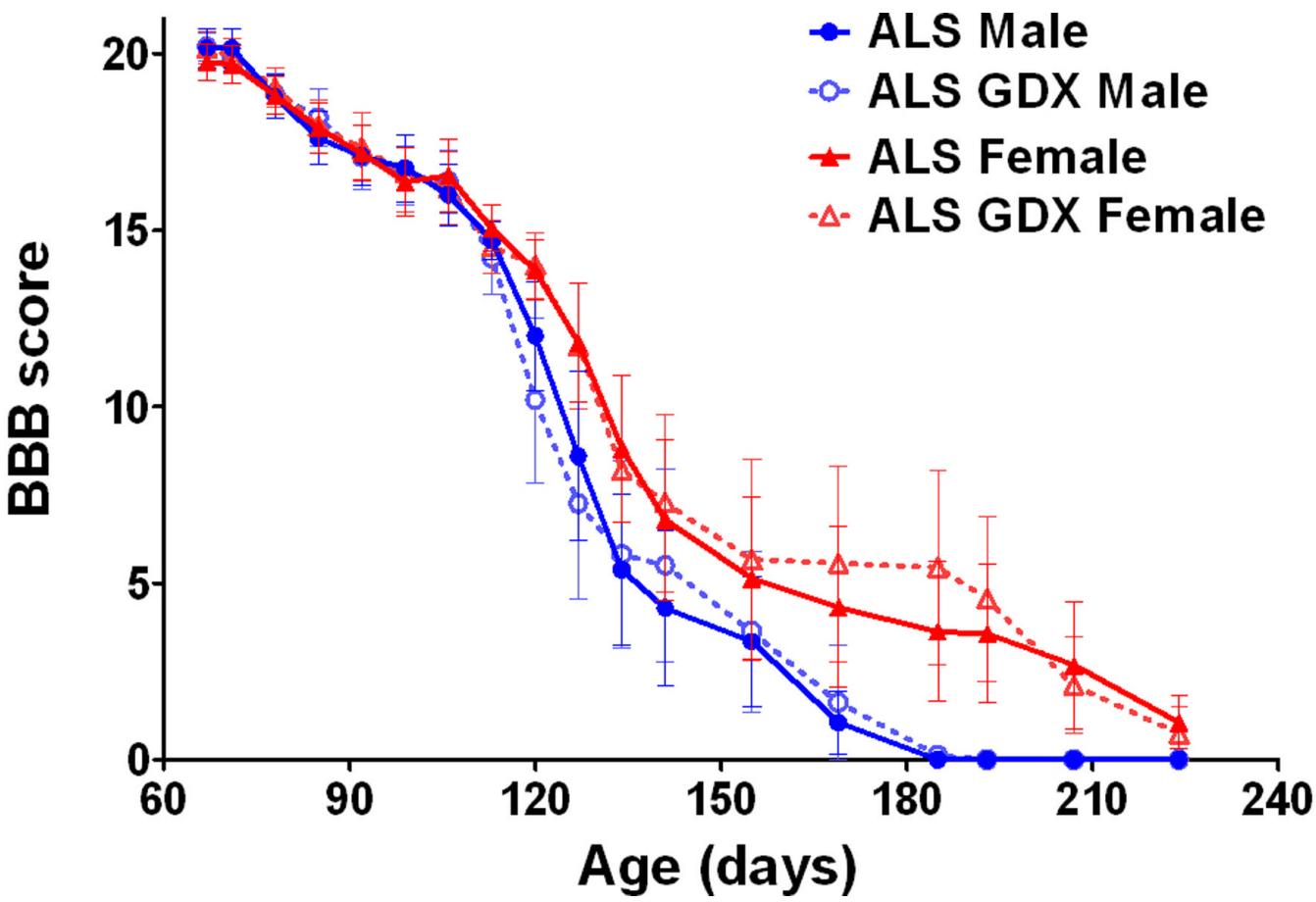

Figure 2. Basso-Beattie-Bresnahan (BBB) locomotor rating scales in gonadectomized SOD1^{G93A} rats

While clear sexual dimorphism was confirmed, gonadectomy did not modulate motor function in both sexes.

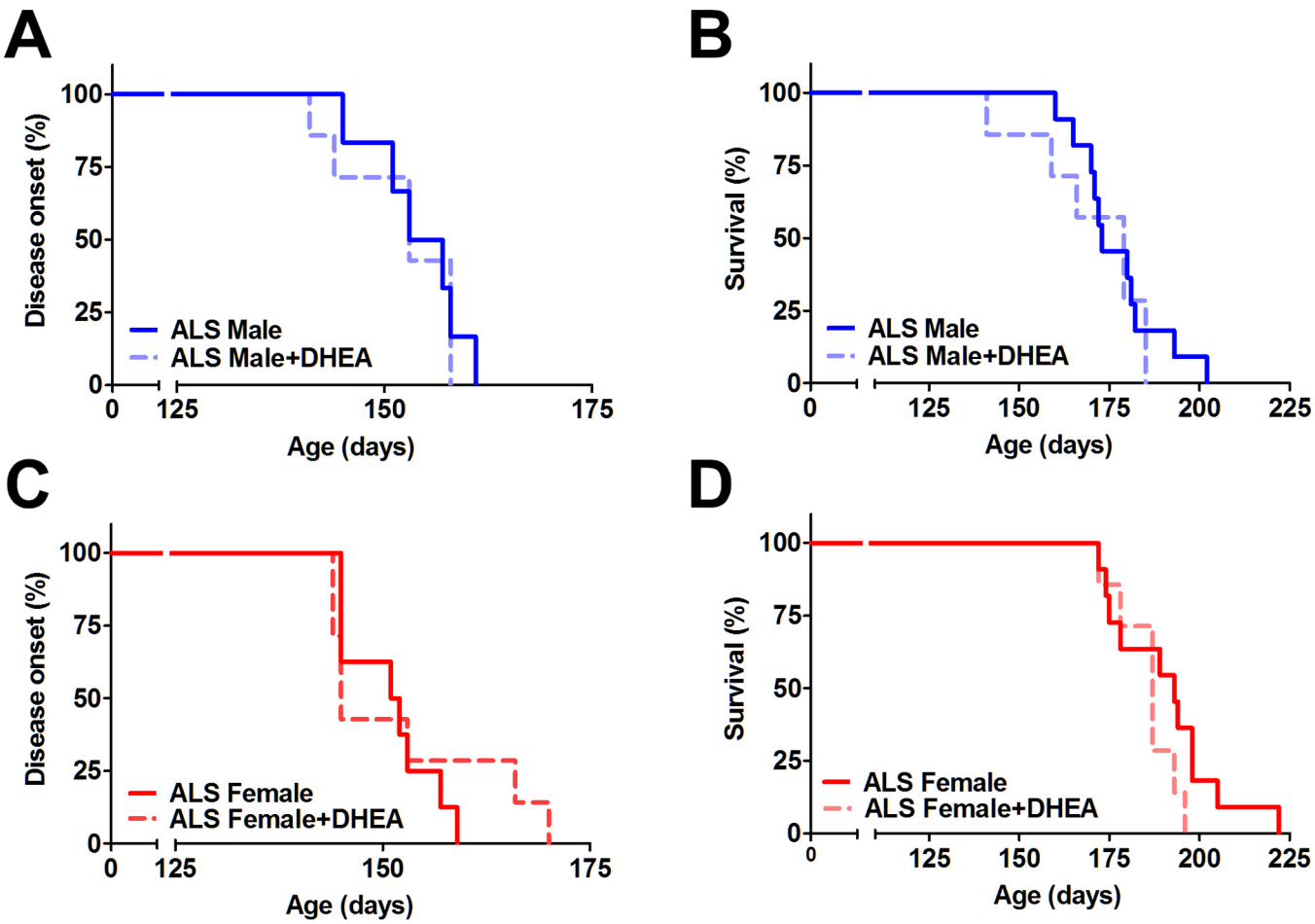

Figure 3. Effects of DHEA treatment in SOD1^{G93A} rats

Chronic DHEA treatment does not alter disease onset and survival period in SOD1^{G93A} males (A, B) and females (C, D).

Table 1

Disease onset and survival period in gonadectomized SOD1^{G93A} rats.

Group	Sex	Disease onset, (days)	Survival (days)
Control SOD1^{G93A}	Male	97 ± 5.1	141 ± 6.3
	Female	104 ± 9.6	161 ± 12.1 *
Gonadectomized SOD1^{G93A}	Male	100 ± 8.3	142 ± 8.0
	Female	101 ± 6.5	162 ± 13.2 **

* P<0.05 vs. control male;

** P<0.05 vs. gonadectomized male.