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The effect of the DcpS inhibitor D156844 on the protective action of follistatin in mice with spinal muscular atrophy

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Abstract

Spinal muscular atrophy (SMA), a leading genetic cause of pediatric death in the world, is an early-onset disease affecting the motor neurons in the anterior horn of the spinal cord. This degeneration of motor neurons leads to loss of muscle function. At the molecular level, SMA results from the loss of or mutation in the *survival motor neuron 1* (*SMN1*) gene. The number of copies of the nearly duplicated gene *SMN2* modulates the disease severity in humans as well as in transgenic mouse models for SMA. Most preclinical therapeutic trials focus on identifying ways to increase *SMN2* expression and to alter its splicing. Other therapeutic strategies have investigated compounds which protect affected motor neurons and their target muscles in an SMN-independent manner. In the present study, the effect of a combination regimen of the *SMN2* inducer D156844 and the protectant follistatin on the disease progression and survival was measured in the SMNΔ7 SMA mouse model. The D156844/follistatin combination treatment improved the survival of, delayed the end stage of disease in and ameliorated the growth rate of SMNΔ7 SMA mice better than follistatin treatment alone. The D156844/follistatin combination treatment, however, did not provide additional benefit over D156844 alone with respect to survival and disease end stage even though it provided some additional therapeutic benefit over D156844 alone with respect to motor phenotype.

Keywords: Motor neuron disease; 2,4-Diaminoquinazoline; Follistatin; Spinal muscular atrophy; Preclinical drug trial; Neonatal mouse; D156844

1. Introduction

Spinal muscular atrophy (SMA), a leading genetic cause of infant death worldwide, is an autosomal recessive degenerative disease characterized by selective loss of α motor neurons of the anterior horn of the spinal cord [1]. As a result of this loss, limb and trunk muscles atrophy. SMA results from the loss or mutation of the *SMN* (survival motor neuron) gene [2]. In humans, there are two *SMN* genes (*SMN1* and *SMN2*) which arose from gene duplication differing by a single C-to-T transition within an exon splice enhancer of exon 7 [3,4]. The *SMN1* transcripts contain exon 7 to produce full-length SMN protein while most of the transcripts produced from *SMN2* lack exon 7 and yield an unstable protein known as SMNΔ7. The copy number of *SMN2* modifies the severity of SMA phenotype in humans [5–7] as well as in transgenic mouse models for SMA [8–10]. *SMN2*, therefore, is a genetic modifier of SMA phenotype.

Numerous studies have identified many types of compounds that increase *SMN2* expression [11]. C5-substituted 2,4-diaminoquinazolines (2,4-DAQs) are potent inducers of *SMN2* promoter activity that were initially identified through a high-throughput drug screen [12]. D156844, a piperidine 2,4-DAQ derivative, increases SMN expression in cultured fibroblasts derived from an SMA patient and ameliorates the survival and phenotype of SMNΔ7SMA mice [13–15]. RG3039, another potent 2,4-DAQ, increases the mean lifespan in different mouse models of SMA [16,17]. 2,4-DAQs are potent inhibitors of the mRNA decapping enzyme DcpS [18].

Administration of recombinant follistatin to SMNΔ7 SMA mice increases the mean lifespan by about 30% [19]. Even though follistatin increases the mean lifespan of SMNΔ7 SMA mice, the maximum lifespan is not affected by this treatment suggesting that follistatin prevents earlier deaths in these mice. Follistatin does not affect SMN expression in the spinal cord or in the skeletal muscle of SMNΔ7 SMA mice suggesting that it exerts its ameliorative effect in an SMN-independent manner [19].

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In the development of an effective therapeutic strategy for SMA, a combinatorial approach has been suggested wherein different classes of therapeutic agents are administered to elicit a multi-faceted protective effect on SMA patients. It is especially desirable to develop a cocktail of therapeutic agents that targets multiple mechanisms underlying SMA pathology, i.e. increasing *SMN2* expression and protection of the motor unit – the motor neuron and the muscles it innervates. The effect of the 2,4-DAQ D156844 on the protective response of SMNΔ7 SMA mice to follistatin was examined in this study.

2. Materials and methods

2.1. Animals and ethical statement

SMNΔ7 SMA mice (*SMN2*$^{+/+}$; *SMNΔ7*$^{+/+}$; *mSmn*$^{-/-}$) were generated from male and female carrier mice (*SMN2*$^{+/+}$; *SMNΔ7*$^{+/+}$; *mSmn*$^{+/-}$) [20]. Since maternal diet influences the survival of SMNΔ7 SMA mice [21], the breeder mice were provided with *ad libitum* water and PicoLab20 Mouse diet (#5058; Purina) rodent chow. Only SMA and carrier pups were used in these experiments. All experiments were conducted in accordance with the protocols described in the National Institutes of Health *Guide for the Care and Use of Animals* and were approved by the Ohio State University Institutional Laboratory Animal Care and Use Committee.

2.2. Drugs

D156844 ([5-(1-(2-fluorobenzyl)piperidine-4-ylmethoxy] quinazoline-2,4-diamine dihydrochloride) was synthesized by deCODE chemistry as described previously [13]. D156844 was dissolved in ddH_2O at a concentration of 3 mg/mL. Recombinant human follistatin (Biovision, Mountain View, CA) was reconstituted in sterile ddH_2O at a concentration of 100 μg/mL.

2.3. Drug administration

Carrier and SMA littermate mice were divided into 3 treatment cohorts: 1) D156844 (3 mg/kg/d) and follistatin (1 mg/kg/qad (every other day)), 2) follistatin alone and 3) ddH_2O. Mice were dosed daily with D156844 or vehicle via oral administration as described previously [22] while follistatin was administered intraperitoneally. Treatment began at postnatal day 4 (PND04) and continued for the lifetime of each SMA mouse. The body mass of each mouse was determined each day during treatment. The treatment cohorts were not stratified based on sex because there is no significant difference in lifespan between male and female SMNΔ7 SMA mice [23] and there are no sex-related differences in the responsiveness of SMNΔ7 SMA mice to D156844 [14].

2.4. Phenotype assays

A cohort of SMNΔ7 SMA mice from each treatment group was assayed for changes in righting reflex success and latency, spontaneous locomotor activity and pivoting activity as described previously [14,23]. Righting reflexes were assessed on PND07 and PND11 while spontaneous locomotor activity and pivoting were monitored on PND07, PND11 and PND14. To minimize the stress on the pup, the spontaneous locomotor activity and pivoting tests were conducted simultaneously.

2.5. Statistical analysis

Data are expressed as means ± standard errors. Kaplan–Meier curves were generated from the survival and onset of body mass loss data and tested using the Mantel–Cox log rank test. The mice in the 3 treatment groups were also compared against previously published, diet-matched D156844 data [15]. All statistical analyses were performed with SPSS v.22.0.

3. Results

3.1. Effect of D156844 and recombinant follistatin on the survival of SMNΔ7 SMA Mice

Consistent with previous findings [19], treatment of SMNΔ7 SMA mice with follistatin ($n = 20$) resulted in a 12% increase in mean lifespan when compared to vehicle-treated ($n = 17$) mice (Fig. 1; 16.1 ± 0.5 days (d) vs. 14.4 ± 0.6 d; $\chi^2 = 4.320$, $p = 0.038$). SMNΔ7 SMA mice ($n = 23$) treated with both D156844 and follistatin exhibited a 20% improvement in mean survival relative to vehicle-treated mice (Fig. 1; 17.3 ± 0.6 d vs. 14.4 ± 0.6 d; $\chi^2 = 9.502$, $p = 0.002$). When comparing the mean survival of SMNΔ7 SMA mice treated with D156844 and follistatin with those treated with follistatin alone, there was no statistically significant difference between these two treatment groups ($\chi^2 = 2.781$, $p = 0.095$). There was, however, a 20% increase in median lifespan in D156844/follstatin-treated SMNΔ7 SMA mice when compared to SMNΔ7 SMA mice treated with follistatin alone (Table 1). Furthermore, the maximum lifespan of SMNΔ7

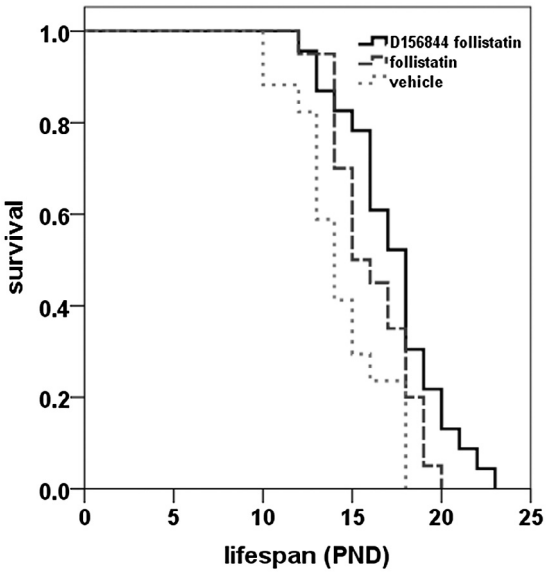

Fig. 1. Oral administration of D156844 augmented the follistatin-induced increase in survival of SMNΔ7 SMA mice. Kaplan–Meier survival plot for SMNΔ7 SMA mice receiving either vehicle (light gray dotted line; $n = 17$), 1 mg/kg/qad* follistatin (gray dashed line; $n = 20$) or a combination of 3 mg/kg/d D156844 and 1 mg/kg/qad follistatin (solid line; $n = 23$). Treatment of SMNΔ7 SMA mice began at PND04. **quaque altera die* (every other day)