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Prediction of death in the SMNΔ7 mouse model of spinal muscular atrophy: Insight into disease stage and progression

Bassem F. El-Khodor[a,*], Kim Cirillo[a], Jose A. Beltran[a], Richard Mushlin[a], Margaret L. Winberg[b], Rachel Charney[a], Olga Chomicova[a], Tara Marino[a], Sylvie Ramboz[a]

[a] PsychoGenics Inc., 765 Old Sawmill River Road, Tarrytown, NY 10591, United States
[b] Spinal Muscular Atrophy Foundation, 888 Seventh Ave, Suite 400, New York, NY 10019, United States

HIGHLIGHTS

- We developed a score that predicts death in the SMNΔ7 mouse model of SMA.
- We showed that SMNΔ7 mice has increased tissue lactate and decrease in respiratory rate.
- The novel score correlates linearly with tissue lactate levels and respiratory rate.
- This work provides insight into the disease stage and a possible biomarker for SMA.
- This method has broad application to various neonatal animal models of neurological disorders.

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ABSTRACT

Proximal Spinal Muscular Atrophy (SMA) is a debilitating neuromuscular disease and a leading inherited genetic cause of infant death. To date, there is no effective treatment for SMA. The SMNΔ7 neonatal mouse model of SMA recapitulates key features of the severe form of SMA and remains a valuable tool in preclinical drug discovery. At any particular postnatal age (*P*), the disease progression in the SMNΔ7 mouse model is not universal, as some animals die as early as the day of birth and others live for up to three weeks. Identification of the disease stage in SMNΔ7 mice, independent of age, would aid in the design and interpretation of preclinical studies. We developed a score (CD score), derived from body weight analysis, that allowed us to gain insight into the disease progression and predict death. Respiratory complication is a leading cause of mortality in the SMA patient and this phenotype has been reported in severe mouse models of SMA. We subsequently measured muscle and brain tissue lactate levels, an indirect measure of hypoxia, in SMNΔ7 mice at P10 and correlated these measures to respiratory rate. SMNΔ7 mice showed a significant increase in tissue lactate and a decrease in respiratory rate in comparison to control. The CD score correlates linearly with tissue lactate level and respiratory rate. The finding of lactate buildup in the SMNΔ7 mouse and the correlation with a score that is predictive of disease stage provide an interesting insight into the disease pathophysiology and a possible biomarker for SMA.

1. Introduction

Proximal Spinal Muscular Atrophy (SMA) is an autosomal recessive neuromuscular disorder caused by a deficiency in survival motor neuron (SMN) protein, resulting from loss or mutation of the survival motor neuron 1 (SMN1) gene. Key features of human SMA are modeled by the deletion of the mouse gene and insertion of human SMN2 transgene and a transgenic cDNA corresponding to the major aberrant isoform of the SMN2 gene (SMNΔ7). The SMN2$^{+/+}$; SMNΔ7$^{+/+}$; Smn$^{-/-}$ mice (SMNΔ7 mouse) have a mean survival of 12.6 ± 0.7 days and show deficiencies in body weight and motor function as early as postnatal day (*P*) 8 (Le et al., 2005). The SMNΔ7 mouse has been characterized (Butchbach et al., 2007a; El-Khodor et al., 2008) and is currently used in preclinical studies (Avila et al., 2007; Bosch-Marce et al., 2011; Butchbach et al., 2010; Foust et al., 2010; Narver et al., 2008; Nizzardo et al., 2011; Riessland et al., 2011; Sumner et al., 2009). The SMNΔ7 mice die between P0 and P18 suggesting that SMNΔ7 mice at any particular postnatal day are not at the same stage of the disease. This poses interpretational challenges for drug efficacy studies using only postnatal age as enrollment criteria. Identification of the disease stage and the approach of death for individual animals, independent of age, would aid in the design and interpretation of efficacy

* Corresponding author. Present address: Pfizer Inc., 500 Arcola Road, N-2144A, Collegeville, PA 19426, United States. Tel.: +1 484 8659072; fax: +1 484 8659391.
E-mail address: bassemfouad.el-khodor@pfizer.com (B.F. El-Khodor).

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and histopathology studies and support decisions to humanely euthanize moribund animals.

One approach toward an in vivo identification of the disease stage is to develop a score that would reliably predict death and correlate with behavioral and biological markers relevant to the disease. We applied advanced body weight analysis relevant to the neonate period and developed a mathematical model for death prediction in SMNΔ7 mice. Although the exact cause(s) of death in the SMNΔ7 mouse remain(s) unknown, it is suspected that respiratory complications may play a role in the demise of these animals. Respiratory complications remain a leading cause of mortality in patients with severe SMA (Giannini et al., 2006; Hardart et al., 2002; Kaindl et al., 2008; Oskoui et al., 2007) and are reported in mouse models of severe SMA (Butchbach et al., 2007a; El-Khodor et al., 2008; Michaud et al., 2010). We measured respiratory rate and tissue lactate levels, an indirect measure of hypoxia, in SMNΔ7 mice at P10 as previously described (El-Khodor and Boksa, 1997). The SMNΔ7 mice showed a significant increase in tissue lactate and decrease in respiratory rate. These two measures showed significant correlation with the death predictor. Sodium Dichloroacetate (DCA), a compound used as a therapeutic intervention in lactate acidosis, reversed the observed lactate accumulation but failed to extend survival, improve body weight or motor function deficiencies, in comparison to controls.

The mathematical model presented in this study allowed us to define the disease stage, predict death, and gain insight into the disease progression in the SMNΔ7 mouse. This work provides a valuable tool for the ongoing preclinical studies in the SMNΔ7 mouse model of SMA as well as to other animal models of human pediatric neurological disorders.

2. Materials and methods

2.1. Animals

Female and male $SMN2^{+/+}$; $SMN\Delta7^{+/+}$; $Smn^{+/-}$ (heterozygote knockout for Smn gene, HET) mice were purchased from Jackson Laboratory, Bar Harbor, Maine, USA (FVB.Cg-Tg(SMN2*delta7) 4299Ahmb Tg(SMN2)89Ahmb Smn1tm1Msd/J, stock number 005025) and were bred to generate a self-sustaining colony of $SMN2^{+/+}$; $SMN\Delta7^{+/+}$; $Smn^{+/-}$ breeder mice. The breeders then generated the $SMN2^{+/+}$; $SMN\Delta7^{+/+}$; $Smn^{-/-}$ (SMNΔ7, homozygote knockouts for Smn, KO), as well as the $SMN2^{+/+}$; $SMN\Delta7^{+/+}$; $Smn^{+/+}$ (wild type for Smn gene, WT) and $SMN2^{+/+}$; $SMN\Delta7^{+/+}$; $Smn^{+/-}$ (heterozygote for Smn gene, HET) control mice. All mice included in the present study were thus homozygous for human $SMN2^{+/+}$ and $SMN\Delta7^{+/+}$ and therefore we will refer to the three genotypes studied as WT, HET and KO based on the status of the mouse Smn gene. One male was housed with 1 and 2 female mice for three days or until vaginal plugs were observed. Pregnant females were housed individually in Opti® cages and were provided with nesting materials, *Envirodri*® bedding, and enriched environments containing plastic igloos and flexible gnaw bones. Food and water were available ad libitum. All mice were maintained at a temperature of 21 °C on a 12 h light/dark cycle. All studies were approved by an Institutional Animal Care and Use Committee (IACUC) established at PsychoGenics Inc., according to the guidelines set out by the Public Health Service, Office of Laboratory Animal Welfare and followed the Guide for Care and Use of Laboratory Animals, 7th edition.

In the present study we utilized KO and WT animals produced over the course of three years, concomitant with ongoing efficacy studies. At birth (defined as P0), litters were culled to 10 pups while keeping equal numbers of males and females (when possible) without prior knowledge of Smn status. Pups were tattooed using non-toxic ink applied under the skin and a tail snip sample was taken for genotyping. Genotyping of the Smn gene was performed by Transnetyx Inc., with data available in 48 h. As needed, litters were further culled to a maximum of 8 pups by removing the extra HET and WT animals at P3. Litters with fewer than 6 pups at P3 were voided, thus litter size used ranged from 6 to 8. Body weight and survival were monitored daily. Two groups of KO animals were evaluated: (1) untreated KO ($N = 100$; 47 males: 53 females) and (2) water treated KO animals ($N = 379$; 192 males: 187 females). Treated KO animals received injections of sterile water (at a dose volume of 5 mL/kg) either once (in the AM), twice (in the AM and PM with at least 4 h between the two doses) or three times per day (AM, noon and PM with at least 3 h between the doses) via oral gavage (PO) starting at P3 and continued until the KO pups died. The PO method applied in this study is a modification of the previously published method by Butchbach et al. (2007b). Using Hamilton® syringes fitted with gavage needles (24 gauge), KO animals at P3 to P4 were given sterile water injections orally via suckling. From P5 to P6, KOs received hemi-oral gavage injection (hPO; a partial oral gavage where the liquid is injected into the mid-esophagus) and from P7 and older, a full oral gavage (PO: liquid injected into the stomach) was given. This method is designed to avoid possible adverse events such as trauma to the trachea and esophagus associated with standard oral gavage at early postnatal days. The shift to full oral gavage as early as P7 is a necessary precautionary measure to avoid possible aspiration in a model with progressive muscle weakness and paralysis. The dose volume for all treatments was 5 mL/kg. Daily body weight for a group of untreated WT littermates ($N = 122$: 63 males, 59 females) was taken during the study to establish a body weight growth profile of the WT control neonates with the same genetic background as the KO animals. Finally, body weights for extra WT and HET littermates were taken at P10, P12 and P14 to monitor the litter and dam overall health.

2.2. Genotyping

Genotyping from tail biopsies was performed by Transnetyx Inc. (Cordova, TN, USA) using a qPCR based probe hybridization method to detect the presence of a neomycin cassette insertion as well as the presence of a disruption in the Smn1 gene sequence at the insertion site.

2.3. Sodium Dichloroacetate (DCA)

DCA (Cat# B21827) was purchased from Alfa Aesar, Ward Hill, MA, USA and was formulated in sterile water (vehicle). Since DCA powder is hygroscopic, the parent vial was opened once and aliquots were made. Dosing solutions were prepared fresh once every three days and stored at 4 °C overnight and between each dosing session.

Seven vehicle (sterile water) and 16 DCA-treated KO animals were included. All groups were gender balanced. DCA (at 50 mg/kg) and vehicle treatments were administered once per day in the morning from P3 until death of the animal via PO injection with a dose volume of 5 mL/kg. In the afternoon of P10 and P12, the motor function of all surviving animals was evaluated using negative geotaxis and hind limb suspension test (a.k.a. tube test) as previously described (El-Khodor et al., 2008).

2.4. Whole brain and hind limb muscle lactate

Eleven WT, 10 HET, 15 untreated KO, 9 vehicle-treated KO and 8 DCA-treated KO were included. All groups were gender balanced. DCA (at 50 mg/kg) and vehicle treatments were administered once per day in the morning from P3 to P10 via PO injection with a dose volume of 5 mL/kg. Four hours after the last dose at P10, the respiratory rate was evaluated as previously described (Devalaraja-Narashimha and Padanilam, 2009; Mittal et al., 2009)