SMA_M.2.2.003

Please quote this SOP in your Methods.

Influence of housing, feeding, and handling conditions on SMA mouse performance

SOP (ID) Number	SMA_M.2.2.003
Version	1
Issued	16th December 2011
Last reviewed	17th May 2019
Authors	Arthur Burghes, Department of Molecular and Cellular Biochemistry, The Ohio State University Charlotte Sumner, Department of Neurology, Johns Hopkins School of Medicine
Working group members	Rashmi Kothary (Ottawa Hospital Research Institute) Christine DiDonato (Children's Memorial Hospital Research Center, Northwestern University, Chicago, IL) Michael Sendtner (Institute for Clinical Neurobiology University of Würzburg, Germany) Rafael Yanez (Biomedical Sciences, Royal Holloway-University of London) Daniel Schümperli (Institute of Cell Biology, University of Bern, Switzerland) Veronica Setola (EPFL-Lausanne-Switzerland) Marco Passini (Genzyme Corporation, Cambridge, MA, USA)
SOP responsible	Arthur Burghes
Official reviewer	Charlotte Sumner

TABLE OF CONTENTS

1. OBJECTIVE

The objective of this SOP is to highlight some of the factors that can influence the performance of spinal muscular atrophy (SMA) mice. These variables should be taken into account when designing preclinical therapeutics efficacy experiments. To date, the SMA mouse model that has been studied most extensively for therapeutics testing is the SMAΔ7 mouse [1] (Jackson Laboratory Strain #005025). This mouse line like other severe SMA mouse models has disease onset and death prior to weaning. Most of the guidelines below are derived from experience in this mouse model. Nonetheless, these principles are relevant for other SMA mouse models (including milder models), which will likely be increasingly used in the future.

2. INTRODUCTION

This SOP is written to provide guidance to investigators evaluating the phenotype of SMA mice, particularly those new to the SMA field. Understanding the factors that can influence SMA mice is important when making conclusions about meaningful changes in this phenotype.

SMAΔ7 mice are to date the line of SMA mice most widely used in different laboratories around the world[1]. Multiple published studies have shown that the phenotype of these mice varies somewhat in different laboratories. The average maximal weight gained by SMAΔ7 mice varies between ~3.0 to ~4.5 grams and the median survival varies between ~12.5 to ~17 days (see Table 1)[1-13]. In addition, the phenotype of individual SMA mice within a given colony can vary. It is not unusual for the survival of individual mice to range from 10 to 18 days. Some of the factors that result in this variability can not be easily controlled by the investigator. These include potential differences in SMN expression levels between mice, differences in the intrauterine environment, and differences in the mothering skills of a given dam. Nonetheless, there are other factors that should be considered and at times controlled for during preclinical therapeutic studies in SMA mice.

3. INFLUENCE OF GENETIC BACKGROUND, GENDER AND BIRTH WEIGHT

3.1 Background strain

Investigators must understand the potential influence of the genetic background on the phenotype of SMA mice. For example, when SMAΔ7 mice were initially derived, they consisted of a mix of the C57Bl/6 and FVB/N strains. Although they were backcrossed onto the FVB/N strain, single nucleotide polymorphism analysis indicated that 11% of the genome was still derived from the C57Bl/6 strain[12]. When these mice were further backcrossed to make them

fully congenic on FVB/N, median survival dropped from an average of 17.7 days to 11.4 days. The survival of SMAΔ7 mice also decreases when mice are congenic on a C57Bl/6 background (Cathleen Lutz, Jackson Laboratories, personal communication).

The use of non-congenic mice has the advantage of longer survival as well as being more representative of the mixed genetic background present in the human population. In the non-congenic background, it is preferable to have both an extension of maximum life span and average life span during drug interventions. Congenic strains are useful when a small difference in survival occurs due to less variability. If a drug compound has a small difference in the non-congenic strain, re-testing in a second mouse line is advisable and in this case a congenic strain should be considered.

In any case, the strain composition of the SMA mouse model being used should be reported when publishing results.

3.2 Gender

Gender has not been reported to have a substantial impact on the phenotype of SMA mice except for a trend to earlier weight loss in females compared to males[2, 6]. Nonetheless, gender should be recorded and reported during therapeutic efficacy studies.

3.3 Birth weight

Birth weight has been reported to not be predictive of survival in SMA mice[6]. However, it has also been observed that SMA mice that are underweight compared to their littermates at the start of a therapeutic intervention may not respond as well to the therapeutic[10]. To try and minimize starting weight as a potential variable, some investigators exclude SMA mice from the therapeutic trial whose birth body weight (the first day of life and not later) is greater than 25% reduced compared to the average of the normal littermate body weight for that litter. Also, birth weight can be cross-matched in litters, as described below.

4. DEFINITION OF SURVIVAL

One of the factors that contribute to the differences in survival observed in different laboratories is variations in the definition of survival. Unfortunately, this is often not formally defined in published studies. In some laboratories, SMA mice are euthanized prior to death for humane reasons when they meet endpoints such as complete paralysis, change in skin turgor, or 20-30% loss of the maximum weight achieved. In other laboratories, death is the survival endpoint. These endpoints will be determined in large part by a given institution's animal care committees; however this definition should be consistent throughout the study and clearly

defined when reporting. In our experience, 30% loss of maximum weight achieved for that animal is highly predictive of imminent death.

5. INFLUENCE OF HOUSING CONDITIONS

5.1 Litter size

It has been reported that litter size does not influence the survival of individual SMAΔ7 mice[6]. Overall this is likely the case, however some investigators have observed that with litter size less than 4, SMA mice will feed better and thus have improved weight gain and survival. Similarly, with litter size greater than 10, SMA mice do not compete well for maternal milk and may do poorly. In order to minimize this variable, some investigators only enroll SMA pups in a therapeutic study if they are derived from a litter that contains between 5-10 pups.

Other investigators cull all litters to a predetermined number of pups (such as 6) within the first few days of life. It is recommended that when culling, that both SMA and normal littermate mice are kept in the litter**.** In the non-congenic strain, cross fostering of pups can be easily performed to balance the number of SMA mice in a particular litter. In the FVB/N background this is relatively straight forward: take a pup and role it in bedding of foster mum prior to placing in cage, but avoid excessive handling. By doing this you can equal the number of SMA mice in a litter to be treated with a drug compound. The litter size can be maintained constant at what is desired. While cross fostering is relatively easy in this line of mice, it tends to be more difficult with the C57/Bl6 background, but is still possible.

Removal of the father will prevent the mother becoming pregnant right away and is usually advised by IACUC regulations. However the father does not have to be removed.

5.2 Temperature

SMAΔ7 mice are hypothermic relative to normal littermate mice likely due to poor movement and less time with the mother[2]. At P6, SMA mice have a 1ºC decrease in body temperature, at P10, it is a ~2.5ºC decrease, and by P12, it is a 5ºC decrease. Adequate bedding (completely covering the bottom of the cage) and heating pads (37°C) under the mouse cage and during testing out of the cage may help mitigate this abnormality.

5.3 Diet

Maternal diet has been shown to influence the survival of SMA mice[14]. When mothers are fed a PicoLab20 diet, which has higher fat content, SMA mice lived a median of 2-3 days longer than when mothers were fed a Harlan Tekland 22/5 diet[14].

In the case of the SMAΔ7 mouse model and other severe SMA mouse models, in which disease onset and death are prior to weaning, SMA mice are receiving nutrition via their mother's milk. Access to this nutrition becomes increasingly difficult as the litter grows because SMA mice cannot compete with normal littermates and cannot access nutrition from an increasingly mobile mother. SMA mouse milk spot size (A visual assessment of quantity of milk in the stomach visible through the pup's translucent skin[2]).is equal to normal littermates at P0, P2, and P4, but by P6 and onwards it is progressively decreased.[2] It has also been shown that SMA mice are significantly hypoglycemic relative to normal littermates at P12 [14].

Ancillary nutrition and/or hydration may also be provided directly to SMA mice. This can increase the responsiveness to therapeutic interventions potentially because of increased tolerability to toxic side effects of drugs or enhanced biodistribution of drug to the CNS. For example, trichostatin A (TSA) delivered to SMA mice between P2-P21 resulted in an increase in median survival to 21 days, but when ancillary nutrition was provided with TSA, median survival was increased to 38 days[15]. Ancillary nutrition may take the form of extra soft food at bottom of cage such as moistened food pellets, enriched carbohydrate paste, or fruit. Alternatively, milk formula can be delivered to SMA pups manual feeding[15] or by oral gavage. Ancillary hydration can be delivered by subcutaneous injections of lactated ringers or other fluids[15]. The type of ancillary nutrition and/or hydration, if applied, should be mentioned in the study.

6. INFLUENCE OF HANDLING AND TESTING CONDITIONS

6.1 Timing of therapeutic delivery

Emerging evidence with delivery of small molecules[15], gene therapy[8], and conditional SMA mice[16] suggests that the earlier a therapeutic is delivered to postnatal SMA mice, the better the response. Although therapeutics will likely have different temporal requirements, investigators should design their experiment to try and deliver therapeutic interventions as early as possible at least in severe SMA model mice.

One can also consider prenatal delivery by dosing the pregnant female from embryonic day 15 or later. In some cases this results in a greater extension of survival than postnatal dosing[7]. However, it is still recommended to continue dosing after birth.

6.2 Dosing

Investigators should be aware that drug levels and metabolism may be quite different in neonatal mice compared to adult mice. Thus measurement of drug levels in neonates is recommended to determine whether the correct doses have been reached including in neural tissues. Investigators should be aware that in some cases, drug has been detected in untreated littermates when a SMA pup has been dosed with active drug indicating transfer of drug within

a litter. This probably occurs during maternal grooming of pups. This is a particular issue for orally bioavailable compounds, but has also been described for other compounds (for instance, this is a well known issue in toxicology testing (pesticides, etc), one of the main places were neonatal mice are used). Thus, it is advisable that separate litters receive drug and vehicle.

6.3 Drug delivery methods

Routes of described drug delivery in SMA mice include intraperitoneal injection, subcutaneous injection, intravenous injection, oral gavage, intraspinal, and intraventricular. In the case of intraventricular injection, extreme caution should be used as severe SMA mice have reported cardiac defects, therefore this method of delivery is not recommend if it can be avoided. Although these are all standard delivery methods, in most cases they have been adapted for neonatal animals by use of small gauge needles (33 gauge) and syringes (10-50 µl volume). The reader is encouraged to read the references below for details.

6.4 Experimental readouts

Behavioral tests

When SMA mice are removed from the cage for behavioral testing, they very quickly drop their body temperature further[2]. After 2 minutes away from the cage, SMA mouse body temperature decreases by 1.5ºC and by 4 minutes it is decreased by ~3ºC. This reduction in temperature negatively impacts performance on motor behavioral measures such as hind limb strength measures[2]. The use of a heat pad is recommended to avoid this problem. Investigators must take care regarding the panel of motor behavioral tests, their order of administration, and their duration such that they minimize the time out of the cage. Once a particular sequence of assessments has been determined, this needs to remain constant for all mice and the duration of the experiment. The time of day that testing is performed should remain constant as well as timing of drug delivery.

Survival as endpoint

For survival we would strongly urge the use of Kaplan Meir log rank test, these are conventional and well understood. The alternative is basically a distribution analysis and then odds of distribution analysis. This can be used, but the graphic representation of Kaplan Meir allows straightforward assessment of a number of points i.e. is it the early death points that are rescued with no max extension of life. Therefore we would recommend the use of Kaplan Meir even if alternative analysis is reported. Maximum life span of treated mice should be reported. There are a number of sources for Kaplan Meir analysis i.e. SPSS software and Sigma Plot.

7. APPENDIX

Table 1. Variability of SMAΔ7 mice in different laboratories

Median survival	Max weight achieved (grams)	Citation/Location
12.6 ± 0.7 days	~3.0 g	El-Khodor et al. 2008, Psychogenics
12.9 days	~3.5 g	Corti et al. 2008 (Comi Lab)
13 days	~3.5 g	Le et al. 2005 (Burghes Lab)
13 days	~ 4.0 g	Sumner et al. 2009 (Sumner Lab)
13.5 days	~3.8 g	Heier &DiDonato, 2009 (DiDonato lab)
13-14 days	~4.0 g	Butchbach et al. 2010 & 2007 (Burghes Lab)
15.5 days	~4.0 g	Foust et al. 2010 (Kaspar Lab)
15.8 days	NR	Mattis et al. 2009 (Lorson Lab)
16 days	~4.2 g	Avila et al. 2007, NIH
15-17 days	~4.5 g	Passini et al. 2010, Genzyme
17 days	NR	Kariya et al. 2008, Jackson Labs
17-18 days	NR	Rose et al. 2009 (Lorson Lab)

NR=Not reported

7. REFERENCES

1. Le TT, Pham LT, Butchbach ME, et al. SMNDelta7, the major product of the centromeric survival motor neuron (SMN2) gene, extends survival in mice with spinal muscular atrophy and associates with full-length SMN. Hum Mol Genet 2005;14:845-57.

2. El-Khodor BF, Edgar N, Chen A, et al. Identification of a battery of tests for drug candidate evaluation in the SMNDelta7 neonate model of spinal muscular atrophy. Exp Neurol 2008;212:29-43.

3. Corti S, Nizzardo M, Nardini M, et al. Neural stem cell transplantation can ameliorate the phenotype of a mouse model of spinal muscular atrophy. J Clin Invest 2008;118:3316-30.

4. Sumner CJ, Wee CD, Warsing LC, et al. Inhibition of myostatin does not ameliorate disease features of severe spinal muscular atrophy mice. Hum Mol Genet 2009;18:3145-52.

5. Heier CR, DiDonato CJ. Translational readthrough by the aminoglycoside geneticin (G418) modulates SMN stability in vitro and improves motor function in SMA mice in vivo. Hum Mol Genet 2009;18:1310-22.

6. Butchbach ME, Edwards JD, Burghes AH. Abnormal motor phenotype in the SMNDelta7 mouse model of spinal muscular atrophy. Neurobiol Dis 2007;27:207-19.

7. Butchbach ME, Singh J, Thorsteinsdottir M, et al. Effects of 2,4-diaminoquinazoline derivatives on SMN expression and phenotype in a mouse model for spinal muscular atrophy. Hum Mol Genet 2010;19:454-67.

8. Foust KD, Wang X, McGovern VL, et al. Rescue of the spinal muscular atrophy phenotype in a mouse model by early postnatal delivery of SMN. Nat Biotechnol 2010;28:271-4.

9. Mattis VB, Fosso MY, Chang CW, Lorson CL. Subcutaneous administration of TC007 reduces disease severity in an animal model of SMA. BMC Neurosci 2009;10:142.

10. Avila AM, Burnett BG, Taye AA, et al. Trichostatin A increases SMN expression and survival in a mouse model of spinal muscular atrophy. J Clin Invest 2007;117:659-71.

11. Passini MA, Bu J, Roskelley EM, et al. CNS-targeted gene therapy improves survival and motor function in a mouse model of spinal muscular atrophy. J Clin Invest 2010;120:1253-64.

12. Kariya S, Park GH, Maeno-Hikichi Y, et al. Reduced SMN protein impairs maturation of the neuromuscular junctions in mouse models of spinal muscular atrophy. Hum Mol Genet 2008;17:2552-69.

13. Rose FF, Jr., Mattis VB, Rindt H, Lorson CL. Delivery of recombinant follistatin lessens disease severity in a mouse model of spinal muscular atrophy. Hum Mol Genet 2009;18:997-1005.

14. Butchbach ME, Rose FF, Jr., Rhoades S, et al. Effect of diet on the survival and phenotype of a mouse model for spinal muscular atrophy. Biochem Biophys Res Commun 2010;391:835-40.

15. Narver HL, Kong L, Burnett BG, et al. Sustained improvement of spinal muscular atrophy mice treated with trichostatin A plus nutrition. Ann Neurol 2008;64:465-70.

16. Le TT, McGovern VL, Alwine IE, et al. Temporal requirement for high SMN expression in SMA mice. Hum Mol Genet 2011.