ELSEVIER

Journal of Neuroscience Methods 161 (2007) 285–290

JOURNAL OF NEUROSCIENCE METHODS

www.elsevier.com/locate/jneumeth

Short communication

A novel method for oral delivery of drug compounds to the neonatal SMNΔ7 mouse model of spinal muscular atrophy

Matthew E.R. Butchbach [a,*], Jonathan D. Edwards [a], Kristie R. Schussler [a], Arthur H.M. Burghes [a,b,c,**]

[a] *Department of Molecular and Cellular Biochemistry, College of Medicine, Ohio State University, 363 Hamilton Hall, 1645 Neil Avenue, Columbus, OH 43210, USA*

[b] *Department of Neurology, College of Medicine, Ohio State University, Columbus, OH, USA*

[c] *Department of Molecular Genetics, College of Biological Sciences, Ohio State University, Columbus, OH, USA*

Received 11 October 2006; received in revised form 31 October 2006; accepted 1 November 2006

Abstract

Spinal muscular atrophy (SMA) is a devastating motor neuron disease that is one of the leading genetic causes of infant mortality. Currently, there is no cure for SMA. Mouse models that genetically and phenotypically resemble SMA have been generated and have the potential to be used for the discovery of novel therapeutics. Oral administration is a commonly used mode of drug delivery in humans as well as in rodents. Unfortunately, there is no method of drug delivery that can accurately and reliably deliver drug compounds orally to neonatal mice. In this report, we describe a novel method to orally administer compounds to neonatal SMA mice. Oral delivery to neonatal mice, usually starting at postnatal day 4 (PND04), is both rapid and safe to the pup. Oral delivery of two different commonly used vehicle formulations, distilled water and 2-hydroxypropyl-β-cyclodextrin, does not affect the survival of SMA mice. After oral delivery for 3 days, 5-bromo-2′-deoxyuridine could be detected in the kidneys, brains and spinal cords of treated non-SMA as well as SMA neonatal pups. In conclusion, we have developed a method by which drugs can be safely and reliably administered orally to neural targets of neonatal mice. This approach offers a simple and rapid means by which potential therapeutics for SMA can be identified.

Keywords: Spinal muscular atrophy; Mouse; Neonatal; Drug delivery; Oral administration; Spinal cord

1. Introduction

Proximal spinal muscular atrophy (SMA) is an autosomal recessive degenerative disease characterized by selective loss of α motor neurons in the anterior horn of the spinal cord (Crawford and Pardo, 1996). SMA is one of the leading genetic causes of infant death in the world. SMA results from the loss or mutation of the *SMN1* (survival motor neuron) gene with retention of *SMN2* (Lefebvre et al., 1995). The severity of the SMA phenotype depends on the copy number of *SMN2* and the levels of SMN protein (Coovert et al., 1997; McAndrew et al., 1997; Lefebvre et al., 1997). SMN is ubiquitously expressed and its levels are reduced in all tissues of SMA patients especially in type I, severe patients (Coovert et al., 1997; Lefebvre et al., 1997). The mechanism which accounts for the motor neuron specificity of SMA is presently unclear.

We now have mouse models of SMA with varying degrees of phenotypic severity (reviewed in Butchbach and Burghes, 2004). Unlike humans, mice carry only one SMN gene (*mSmn*) which is equivalent to *SMN1* (DiDonato et al., 1997; Viollet et al., 1997). Loss of *mSmn* results in embryonic lethality in the mouse suggesting that the mSmn gene product is essential for cell function and survival (Schrank et al., 1997). Insertion of *SMN2* into mSmn null mice by transgenesis rescues the embryonic lethality phenotype (Monani et al., 2000). However, mice with low copy numbers (i.e. 1–2) of *SMN2* develop severe (type I-like) SMA and die at 6–8 days (Monani et al., 2000; Hsieh-Li et al., 2000). Introducing SMN lacking exon 7 (SMNΔ7) into *SMN2*; $mSmn^{-/-}$ mice partially ameliorates the SMA phenotype and these mice die at 14–15 days (Le et al., 2005). The *SMN2*;

* Corresponding author. Tel.: +1 614 688 4710; fax: +1 614 292 4118.
** Corresponding author. Tel.: +1 614 688 4759; fax: +1 614 292 4118.
E-mail addresses: butchbach.1@osu.edu (M.E.R. Butchbach), burghes.1@osu.edu (A.H.M. Burghes).

0165-0270/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jneumeth.2006.11.002

SMNΔ7; $mSmn^{-/-}$ mice show that SMNΔ7 is functional but it does not produce enough functional SMN protein to completely rescue SMA-like motor neuron degeneration. Introduction of a missense mutation (SMN(A2G)) found in type III SMA patients (Parsons et al., 1998) into *SMN2*; $mSmn^{-/-}$ mice modulates the SMA phenotype such that the transgenic mice can survive for over 1 year (type III SMA) (Monani et al., 2003).

One of the many potential uses for these mouse models of SMA is the testing of potential therapeutic agents for the amelioration of the neurodegenerative phenotype seen in these mice. A common route of administration of drugs to humans as well as to rodents is oral delivery. In rodents, oral administration is usually accomplished by placing the drug of interest into the water supply. For example, treatment of type II/III SMA-like mice (*SMN2*; $mSmn(\Delta 7)^{-/-}$) with sodium butyrate (0.8–8.0 mg/mL) via the drinking water increased their survival by 4–5 days (29%) (Chang et al., 2001). Delivery of drugs via voluntary consumption of water does not account for individual variability in the amount of water – and, hence, drug – consumed in a given day. To further complicate drug delivery to neonatal mice, the drug which is consumed by the dam via the water supply must be able to accumulate in the dam's milk so that it can delivered to the suckling pups. Administration of drugs *ad libitum* via the water supply, therefore, does not reliably deliver the drug to individual neonatal mice.

Unfortunately, there is no method of minimally invasive drug delivery that can accurately and reliably deliver drug compounds orally to neonatal mice. In this paper, we describe a novel method by which a drug can be safely administered to neonatal mouse pups. This method of oral administration is similar to gavage except that the feeding needle in our method is not delivered to the stomach thereby preventing any potential damage to the esophagus. The effect of various vehicle formulations on the lifespans of SMNΔ7 SMA mice was assessed. Additionally, we determined if a compound – in this case, BrdU – can reach the brain and spinal cord of neonatal pups when administered orally. This method of drug delivery could be used to test the effect of a given drug on the amelioration of the degenerative phenotype observed in SMA mice as well as other mouse models for other early onset neurological disorders.

2. Methods

2.1. Animals

SMNΔ7 SMA mice were generated from males and females of the genotype $SMN2^{+/+}$; $Smn\Delta 7^{+/+}$; $mSmn^{+/-}$ (*FVB.Cg.Tg*(*SMN*delta7)4299Ahmb Tg*(*SMN2*)89Ahmb Smn1*^{tm1Msd}; Le et al., 2005). These mice are on a FVB/N genetic background. Although the mice used in these experiments were derived from our colony, they are available from Jackson Laboratories. Mice were maintained on a 12 h light:12 h dark cycle (light period 06:00 until 18:00) with *ad libitum* access to food and water. All breeding dams were provided with nesting material before parturition and delivered their pups spontaneously. The date of birth was designated as postnatal day (PND) 01. Neonatal offspring were genotyped using a PCR-based assay on genomic DNA from tail biopsies – obtained after death – as described previously (Le et al., 2005). 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. Oral delivery of drugs

Oral delivery of compounds to neonatal pups was accomplished using a modified gavage procedure. A stainless steel, 24-gauge feeding needle (Harvard Apparatus; needle length = 25.4 mm, ball diameter = 1.25 mm) was bent to form a curve as shown in Fig. 1. The mouse pup was gently but securely grasped by the skin on the back of its neck and the feeding needle was gently inserted into the oral cavity just until the needle reached the pharynx (Fig. 2). The drug compound was slowly injected into the oral cavity after which the feeding needle was slowly removed from the mouth. The Supplemental Video shows in detail the approach used to administer a given drug orally. Mice were sometimes lightly sedated with the gaseous anesthetic isoflurane prior to administration of drug so as to minimize any potential harm to the animal (Murphy et al., 2001). Sedation does not affect the mortality of mice receiving oral administration of a drug. Drugs were administered to mice until the last SMA pup in the litter exhibited prolonged periods (>10 min) of lethargy and >20% loss of body mass over a 24-h period.

2-Hydroxypropyl-β-cyclodextrin (HPCD; Sigma–Aldrich) was dissolved in sterile water to a final concentration of 40% (w/v) and filter sterilized into small aliquots. HPCD as well filter sterilized ddH_2O were administered to neonatal pups

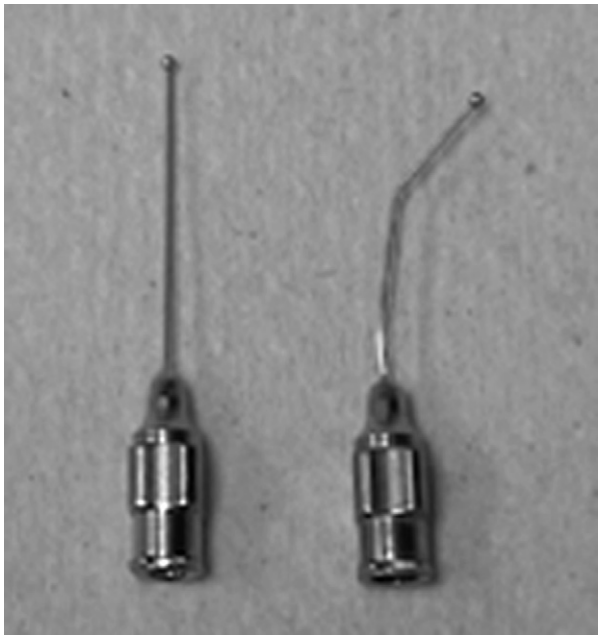

Fig. 1. Feeding needles used in oral delivery of drug compounds to neonatal mice. This photograph shows a stainless steel, 24-gauge feeding needle which has been bent ∼30° (on right) so as to facilitate insertion into the oral cavity. An unbent feeding needle is shown on the left.