Biochemical and Biophysical Research Communications 417 (2012) 376–381

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Direct central nervous system delivery provides enhanced protection following vector mediated gene replacement in a severe model of Spinal Muscular Atrophy

Jacqueline J. Glascock [a,b,1], Monir Shababi [a,1], Mary J. Wetz [a,b], Megan M. Krogman [a,b], Christian L. Lorson [a,b,*]

[a] Department of Veterinary Pathobiology, Bond Life Sciences Center, University of Missouri, Columbia, MO 65211, USA
[b] Department of Molecular Microbiology and Immunology, Bond Life Sciences Center, University of Missouri, Columbia, MO 65211, USA

ARTICLE INFO

Article history:
Received 21 November 2011
Available online 8 December 2011

Keywords:
Survival Motor Neuron (SMN)
Spinal Muscular Atrophy (SMA)
scAAV
Gene therapy
Neurodegeneration
Therapeutics

ABSTRACT

Spinal Muscular Atrophy (SMA), an autosomal recessive neuromuscular disorder, is the leading genetic cause of infant mortality. SMA is caused by the homozygous loss of Survival Motor Neuron-1 (SMN1). SMA, however, is not due to complete absence of SMN, rather a low level of functional full-length SMN is produced by a nearly identical copy gene called SMN2. Despite SMN's ubiquitous expression, motor neurons are preferentially affected by low SMN levels. Recently gene replacement strategies have shown tremendous promise in animal models of SMA. In this study, we used self-complementary Adeno Associated Virus (scAAV) expressing full-length SMN cDNA to compare two different routes of viral delivery in a severe SMA mouse model. This was accomplished by injecting scAAV9-SMN vector intravenously (IV) or intracerebroventricularly (ICV) into SMA mice. Both routes of delivery resulted in a significant increase in lifespan and weight compared to untreated mice with a subpopulation of mice surviving more than 200 days. However, the ICV injected mice gained significantly more weight than their IV treated counterparts. Likewise, survival analysis showed that ICV treated mice displayed fewer early deaths than IV treated animals. Collectively, this report demonstrates that route of delivery is a crucial component of gene therapy treatment for SMA.

1. Introduction

Spinal Muscular Atrophy (SMA) is caused by the homozygous loss of Survival Motor Neuron-1, SMN1 [1,2]. The human genome contains two nearly identical SMN genes, SMN1 and SMN2, however, only SMN1 functions as the disease-determining gene [3,4]. SMN1 and SMN2 differ by a silent C to T transition at the 5′ end of exon 7 [5,6]. This difference alters the alternative pre-mRNA splicing ratios from the two genes, resulting in high levels of full-length product from SMN1, whereas SMN2 produces low levels of full-length SMN and an abundant alternatively spliced isoform, SMNΔ7. The truncated isoform is unstable and cannot compensate for the loss of SMN1 [3]. Despite the ubiquitous expression of SMN, preferential loss of motor neurons occurs in SMA. Because SMA is monogenic, vector-based gene replacement of SMN1 is an attractive option for the treatment of SMA. Encouraging reports have been published using a relatively severe model of SMA called SMNΔ7. These mice lack endogenous mouse *Smn*, but express the human SMN2 gene and the cDNA encoding the alternatively spliced isoform produced by SMN2, SMNΔ7 ($Smn^{-/-}$; $SMN2^{+/+}$; $SMN\Delta7^{+/+}$) [7]. Untreated SMNΔ7 animals live approximately 14 days with disease symptoms becoming overtly apparent around day 7 [7]. Delivery of full-length SMN cDNA to SMNΔ7 neonates using scAAV8 or scAAV9 vectors resulted in significant extensions in survival ranging from an average of 60–200+ days [8–11], with some treated mice displaying a full rescue in terms of lifespan and motor function. However, it remains unclear whether the different injection paradigms or the vector serotype was the primary cause for the differences in the degree of phenotypic rescue.

In this report we utilized a scAAV9-SMN vector and examined two routes of injection in neonatal SMNΔ7 mice [12]. Pups received injections of 2×10^{10} viral genomes via the facial vein (IV) or directly into the brain ventricles (ICV) on postnatal day 2 (PND2). We demonstrate that at this relatively low viral titer, animals receiving ICV injections gained significantly more weight and lived longer than animals receiving IV injections. As expected, animals receiving ICV injections also had higher SMN protein levels in the brain and lumbar spinal cord as compared to IV injected animals. From these results, we conclude that the route of injection for scAAV9-SMN has a significant impact upon the degree of

* Corresponding author. Address: Department of Veterinary Pathobiology, Life Sciences Center, Room 471G, University of Missouri Columbia, MO 65211, USA. Fax: +1 573 884 9395.
E-mail address: lorsonc@missouri.edu (C.L. Lorson).
[1] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2011.11.121

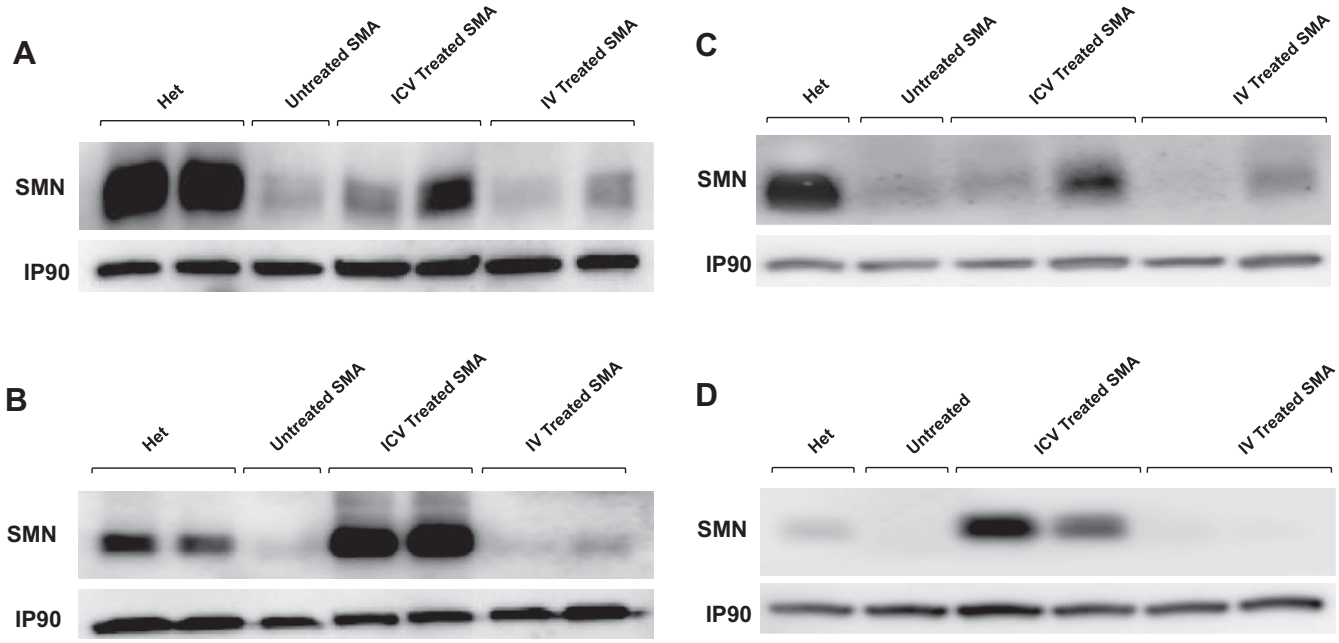

Fig. 1. Western blot showing protein expression is increased to near normal levels following ICV treatment with scAAV9-SMN, while IV treatment results in a more modest increase. Western blots of (A) PND 7 and (B) PND14 brain tissue. (C) PND7 and (D) PND14 spinal cord. All tissues were collected on the respective days from animals injected on PND2 with 2×10^{10} viral particles. Controls were untreated SMA ($Smn^{-/-}$; $SMN2^{+/+}$; $SMN\Delta7^{+/+}$) and unaffected, heterozygous (het) animals ($Smn^{+/-}$; $SMN2^{+/+}$; $SMN\Delta7^{+/+}$).

phenotypic rescue and sheds light upon the development of disease and potential therapeutic implications.

2. Materials and methods

2.1. Genotyping and mouse handling

Animals were handled according to the University of Missouri Animal Care and Use Committee approved Protocols. Mice heterozygous for *mSmn* ($Smn^{+/-}$; $SMN2^{+/+}$; $SMN\Delta7^{+/+}$) were interbred to generate experimental SMA cohorts ($Smn^{-/-}$; $SMN2^{+/+}$; $SMN\Delta7^{+/+}$). The day of birth was counted as PND1 and the neonates were genotyped within 24 h. Animals were genotyped using PCR conditions as previously described [13]. SMA mice were raised with two heterozygous siblings. Additional heterozygous and wild-type animals were culled at the time of injection in experimental cages to control for litter size.

2.2. Tissue collection

Dissections were done as follows: the vertebral column was separated from the torso, then the spinal cord was removed and divided into the cervical, thoracic, and lumbar (C-T-L) regions. The C-T-L sections were immediately frozen. The brain was removed from the skull and divided into four equal sections and each section was immediately frozen. The hindlimbs of each animal were removed at the highest point possible. The foot was bent at a 90° to control for differences in muscle stretching and the tissue was fixed in 4% paraformaldehyde overnight. After fixation, the gastrocnemius and tibialis anterior were removed from the bone, embedded in paraffin, and cross sectioned. The sections were stained with hematoxylin and eosin stain for muscle fiber size analysis. Quantification of fiber size was done as previously described [14].

2.3. Western blotting

Tissues were harvested at indicated times and analysis was performed as previously described [15,16]. Mouse monoclonal anti-SMN (BDBiolabs), 1:2000, and anti-IP90 polyclonal rabbit antibody, 1:2000, were used for SMN and calnexin detection, respectively.

2.4. Production of scAAV-SMN viral vector

scAAV9-SMN was produced via triple transfection in HEK293T cells using polyethyleneimine as previously described [17]. The scAAV plasmid expresses the SMN full-length cDNA (NCBI accession number NM_000344) under the control of the chicken beta actin promoter. Forty eight hours post transfection, the cells were collected and the vector was purified by two cesium chloride density gradient ultracentrifugation steps and dialyzed against HEPES buffer (20 mM HEPES, 100 mM NaCl). Viral particles were tittered by quantitative realtime PCR using SYBR green.

2.5. In vivo injections

Beginning on PND2 $mSmn^{-/-}$; $hSMN2^{+/+}$; $SMN\Delta7^{+/+}$ mice were injected with 2×10^{10} viral particles of scAAV9-SMN. Due to the volume restrictions of the ICV technique, a series of three injections were given to obtain a titer of 2×10^{10}. Animals chosen for ICV injections were injected twice on PND2 (AM and PM) and once on PND3 (AM). Animals chosen for IV injection received a single injection on PND2. Injections were visualized for accuracy by the additional of filter sterilized food dye.

2.6. Motor function analysis

Time to right was measured from p10 to p18. Mice were placed on their backs and given a maximum of 30 s to right themselves onto their paws. Failure to right within 30 s was considered failure. Grip strength and rotarod tests were performed with mice older than 80 days. The rotarod and grip strength assessments were done for 20 consecutive days. The first 10 days served as an initiation and learning period for the animals and the last 10 days were used for analysis. Each individual mouse was given three trials and the best trial was graphed.