Chapter 4

Gene Delivery into the Central Nervous System (CNS) Using AAV Vectors

Koichi Miyake, Noriko Miyake and Takashi Shimada

Additional information is available at the end of the chapter

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Abstract

Application of gene therapies is a promising approach to the treatment of various neurological disorders, including Parkinson's disease, amyotrophic lateral sclerosis (ALS), and lysosomal storage disorders, which are not treatable by any other means. However, the blood–brain barrier (BBB) is a key obstacle to gene delivery to the central nervous system (CNS). Adeno-associated virus (AAV) vectors have emerged as a promising tool for gene delivery to the CNS, thanks to their safety and ability to transduce non-dividing neuronal cells. In this chapter, we discuss strategies for delivering genes across the BBB, focusing especially on potential routes of administration of AAV vectors and promising applications of AAV vectors to the treatment of CNS disorders.

Keywords: Adeno-associated virus vector, central nervous system, routes of administration, lysosomal storage disorders

1. Introduction

Because it is often difficult to treat central nervous system (CNS) disorders using standard pharmacological methods, other, more effective, strategies are being sought. Among these alternatives, gene therapy appears to be a promising approach to treating various neurological disorders, including Parkinson's disease, amyotrophic lateral sclerosis, Huntington disease, Alzheimer's disease, and lysosomal storage disorders, which are not treatable by any other methods. However, the efficacy of gene therapies in clinical trials has been limited by physiological barriers unique to the CNS, as well as by the post-mitotic state of many of the cellular targets in the brain and spinal cord. The blood–brain barrier (BBB) in particular is a key obstacle to gene delivery to the CNS.

Adeno-associated virus (AAV) is a member of the family Parvoviridae that has been widely used as a vector for gene delivery. AAV is a small, non-enveloped single-stranded DNA virus with a genome of approximately 4.7 kb [1]. The AAV genome consists of three open reading frames (ORFs) flanked by two inverted terminal repeats (ITRs). It is a dependovirus because it requires helper functions from other viruses such as adenovirus or herpes simplex virus for its replication. AAV is a suitable gene transfer tool because of its safety due to a lack of pathogenicity, its ability to transduce both dividing and non-dividing cells, and its minor immune response. Among the more than 100 nonredundant AAV genotypes that have been identified, 12 AAV serotypes with unique properties have been used to produce most expression vectors [2].

AAV vectors are powerful tools that are able to mediate gene transfer to the CNS, thanks to their safety and ability to transduce non-dividing neuronal cells. Consequently, they hold great potential for use in therapeutic gene delivery strategies for the treatment of neurological disorders. Although a breakthrough treatment has remained elusive, current approaches are now considerably safer and potentially much more effective than in the past. In this chapter, we discuss how to administer vectors across the BBB, focusing especially on potential routes for administration of AAV vectors and promising strategies for application of AAV vectors in CNS disorders.

2. AAV vectors for CNS disorders

2.1. Advantages of AAV vectors for gene transfer to CNS

A variety of both viral and non-viral vectors have been applied to the effort to transfer genes into the CNS. Among these gene transfer methods, AAV vectors have emerged as a particularly promising tool for gene delivery. There are many advantages to using AAV vectors for transduction of the CNS [3]. First, AAV is itself not pathogenic and has received a P1 and P1A recombinant DNA safety classification. It is therefore easy to use AAV vectors without specific facilities. Second, because AAV vectors have the ability to transduce non-dividing cells, they are a suitable means for delivering transgenes to non-dividing neuronal cells [4]. Third, long-term expression of transgenes with little immunogenicity or toxicity can be achieved using AAV vectors. It was reported that there was a gradual decline in the number of transduced cells when the cytomegalovirus (CMV) immediate-early promoter was used [5]. However, with the use of cellular or hybrid promoters, such as the chicken beta-actin/CMV promoter, transgene expression was sustained. Indeed, we found that following administration to mice of an AAV vector in which the CAG promoter drove the green fluorescent protein (GFP) gene, GFP expression was sustained for more than one and a half years, or nearly the entire life span of the mice [6]. In addition, we are now using several AAV vector serotypes (mainly from AAV serotype 1 to 12), depending on the target. On the other hand, a disadvantage of AAV vectors is the size limitation of the transgene. Since AAVs package a ~4.7-kb genome, it is better to have 4.7 kb between the inverted terminal repeats at the 5′ and 3′ ends [7].

2.2. How to cross the BBB?

To apply gene therapy to the treatment of CNS disorders, there are two immediate problems that must be solved: One is how to cross the BBB, which is a physical and biochemical barrier that precisely regulates the ability of endogenous and exogenous substances to accumulate within brain tissue [8, 9], and the second is how to distribute the transgene to the entire brain. In some cases, we have to treat the whole brain, including the peripheral nervous system (PNS). To transfer genes across the BBB, there are two main approaches: brain-directed local therapy and less invasive systemic therapy. Table 1 shows possible approaches of gene delivery to CNS using AAV vectors.

1. Brain-directed gene transfer
1) Intracranial injection of AAV vector
2. Systemic gene transfer
1) Intravenous injection of AAV vector
2) Intracerebroventricular injection of AAV vector
3) Intrathecal injection of AAV vector

Table 1. Possible approaches of gene delivery to CNS using AAV vectors

3. Local gene delivery to the CNS using AAV vectors

Brain-directed local injection of AAV vectors is a straightforward approach to gene transfer to the CNS. We compared the transduction efficiency of several AAV vector serotypes encoding the luciferase gene (AAV/Luc) after intracranial injection in mice. Fig. 1 shows the resultant transduction efficiencies determined using an in vivo imaging system (IVIS). Efficient transduction was achieved using the AAV9/Luc or AAV10/Luc vectors compared to the AAV1/Luc or AAV8/Luc vectors, and sustained expression was detected for at least 6 months after injection. Notably, however, following injection of a small amount of AAV1/Luc or AAV9/Luc vectors (2 μl) into the striatum, expression of the transgene was detected in the liver after 2 weeks of injection (Fig. 1A). Therefore, although expression of the transgene was absent at 6 months after injection, one must be aware of the potential for the occurrence of unexpected transduction following directed local injection of an AAV vector.

Fig. 2 shows another comparison of transduction efficiency after local administration to the CNS, this time using AAV vectors encoding GFP (AAV/GFP). As expected, the AAV9/GFP vector exhibited the greatest ability to transduce neuronal cells 2 weeks after injection. Surprisingly, however, nearly the same high transduction efficiency was detected 2 months after injection of the AAV2/GFP vector. Although a long time is needed to achieve strong expression with AAV2, since there is no limitation for the patient, the use of AAV2 is one option for highly efficient CNS transduction.

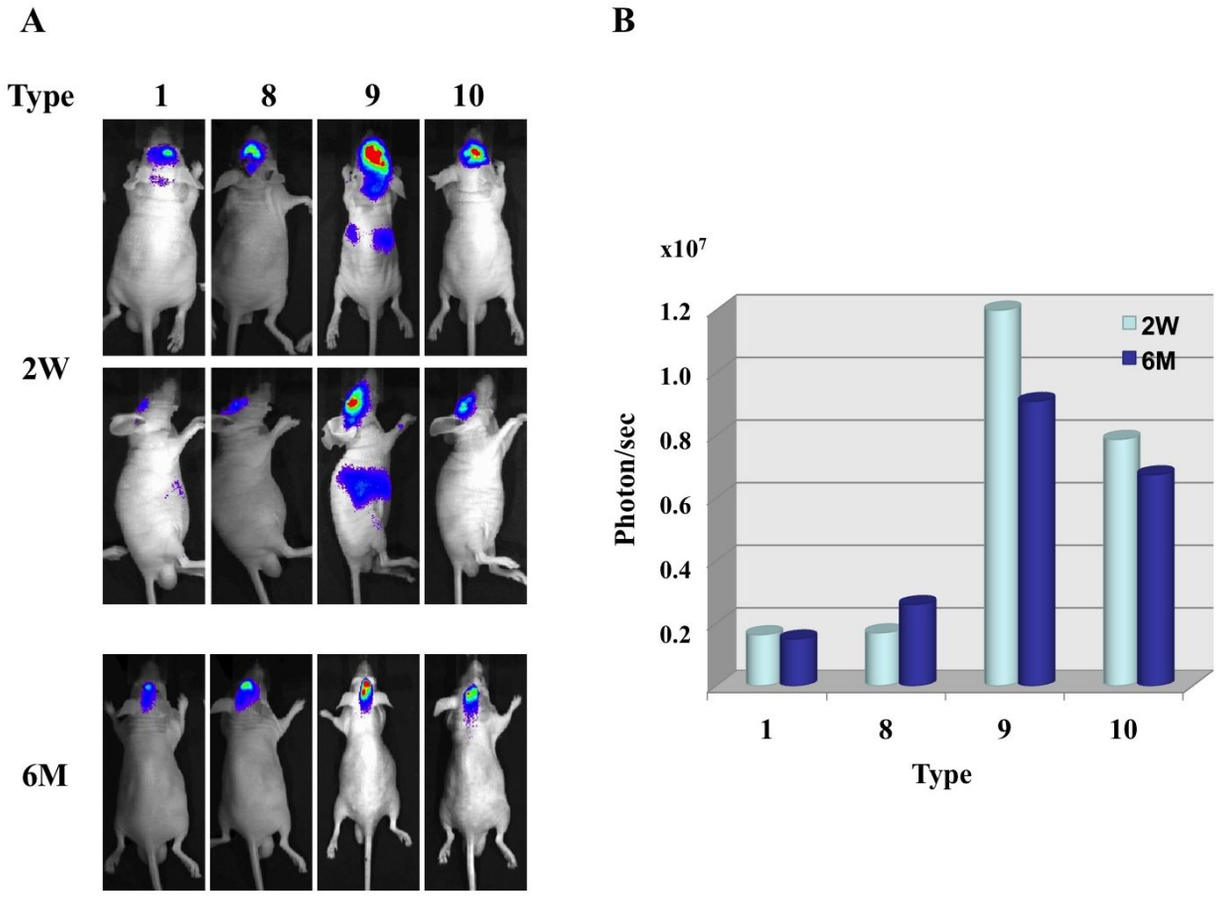

Figure 1. Brain-directed injection of AAV vectors encoding the luciferase gene (AAV/Luc). (A) Approximately 2.0 x 10^{10} vector genomes (vg) of recombinant AAV/Luc vectors (serotypes 1, 8, 9, and 10) were injected into the right striatum over a period of 5 min using a Hamilton syringe with a 33-G blunt-tip needle. Bioluminescent images of mice were obtained using a Xenogen IVIS imaging system at 2 weeks and 6 months post administration. (B) Comparative measurement of AAV/Luc transduction *in vivo* in the brain area 2 weeks and 6 months after injection.

Finally, Fig. 3 shows results obtained with direct intracranial injection of AAV1/GFP vectors into the hippocampus (CA3). Although we injected AAV1/GFP vectors into the right hippocampus (CA3), GFP expression was detected on both sides of the brain, indicating that GFP is efficiently transported to the left side through long axons. This axonal transport is an advantage of direct injection [10, 11].

4. Systemic gene delivery to CNS using AAV vectors

Although in some cases axonal transport may be useful for widespread transduction with AAV vectors, most often local injection of AAV vectors provides transgene expression only to limited regions in the CNS. Consequently, to obtain global transduction of the CNS, multiple intracerebral injections are needed. But this strategy is invasive, and safety becomes a problem. To overcome this problem, an ideal approach for efficient and safe transduction to CNS is systemic administration.

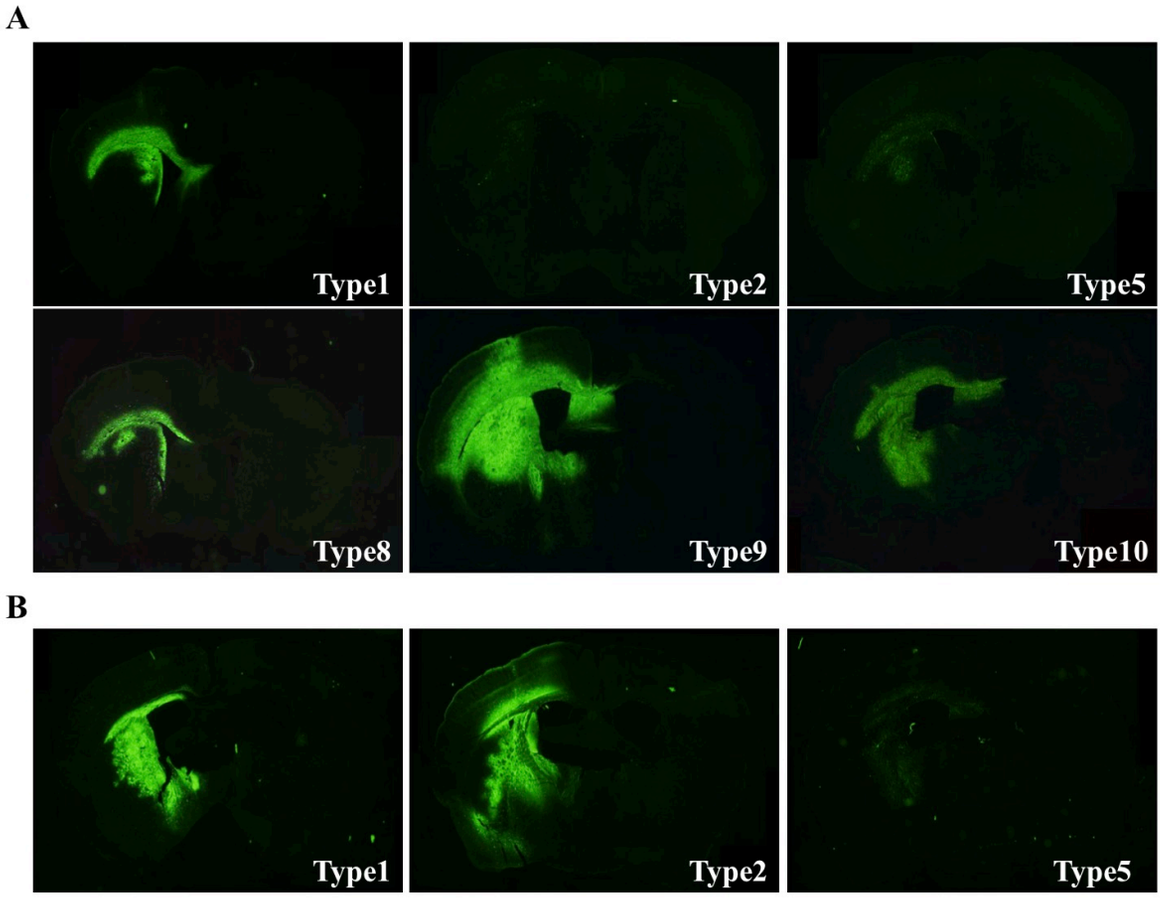

Figure 2. Brain-directed injection of AAV vectors encoding green fluorescent protein gene (AAV/GFP). Approximately 2.0 x 10^{10} vg of AAV/GFP vectors (serotypes 1, 2, 5, 8, 9, and 10) were injected into the right striatum over a period of 5 min using a Hamilton syringe with a 33-G blunt-tip needle. Expression of GFP was analyzed using fluorescent microscopy at 2 weeks (A) and 2 months (B) post administration.

4.1. Systemic administration of AAV vectors for gene transfer to CNS

4.1.1. Systemic administration of AAV vectors during the neonatal period

Systemic administration of AAV vectors is a promising approach for widespread organ transduction, though the BBB is an obstacle to the transduction of the CNS. To overcome this problem, one possibility is to administer the vector during the neonatal period, when the BBB is immature. We injected AAV/GFP vectors (serotypes 1, 8, 9, and 10: 1.5 x 10^{11} vg each) into the jugular veins of neonatal mice and then used diaminobenzidine (DAB) staining to examine GFP expression. GFP signals were detected throughout the entire brain after injection of any of these serotypes. Efficient gene transfer was obtained by AAV9/GFP or AAV10/GFP vector injection (Fig. 4A). Fig. 4B shows immunohistochemical staining of GFP in the brain by systemic neonatal injection of AAV9/GFP vectors. GFP expression was detected throughout the brain, including the olfactory bulb, cerebral cortex, hippocampus, and brainstem, and the spinal cord was also transduced efficiently. However, after the use of the AAV8/GFP vector, widespread transduction in the brain was detected 2 weeks after injection. Moreover, global

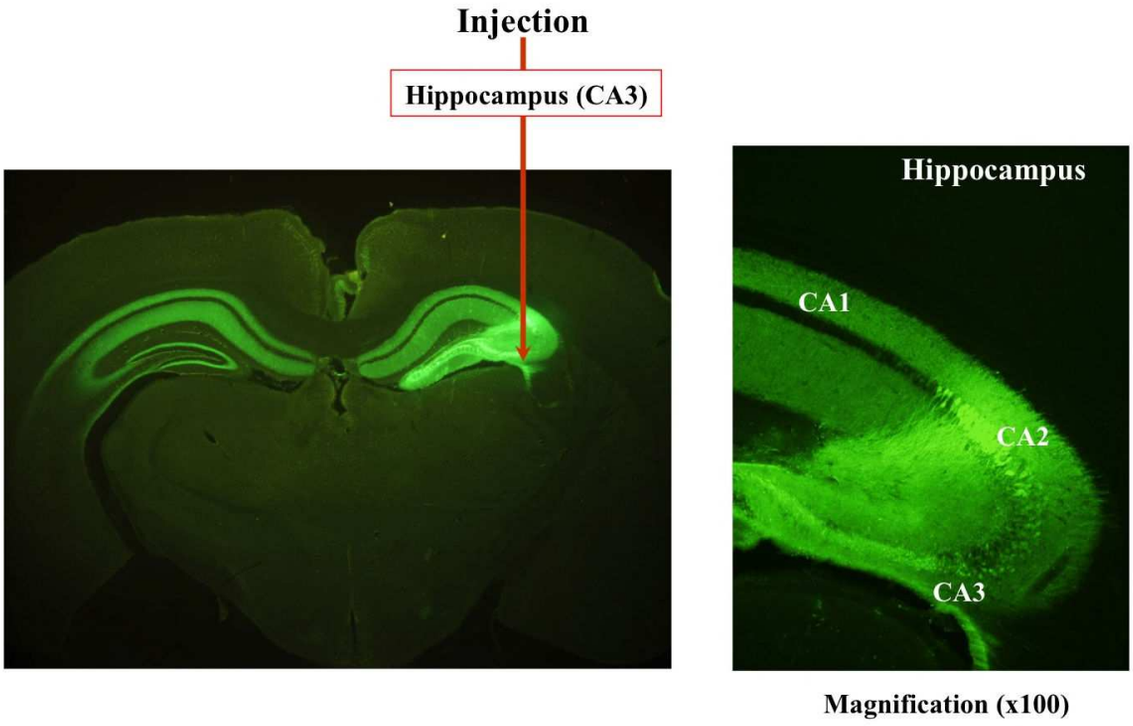

Figure 3. Brain-directed injection of AAV1/GFP vectors into hippocampus (CA3). The CA3 regions of the hippocampus of 7-month-old mice were injected with AAV1/GFP vectors (8.0×10^9 vg) and examined 5 months later. Using a fluorescent microscope, slices of the hippocampal regions were analyzed for GFP expression.

expression of GFP was sustained for at least 18 months (Fig. 4C). Immunohistochemical staining revealed the presence of GFP within GFAP-positive astrocytes, NeuN-positive neurons, and Calbindin-positive Purkinje cells [6]. These findings suggest that systemic neonatal administration of AAV is an effective means of delivering transgenes to target neuronal systems.

4.1.2. Systemic administration of AAV vectors after the neonatal period

It is our experience that AAV vectors are able to pass through the BBB for at least 2 weeks after birth, but within 6 weeks, all AAV vectors lose the ability to cross the BBB [6]. Therefore, to transduce the CNS of adult mice, double-stranded (or self-complementary) AAV vectors (dsAAV) must be used [12]. When we injected single-stranded (ss) AAV9 or dsAAV9 vectors encoding GFP into the tail veins of 8-week-old mice and assessed GFP expression immunohistochemically, minimal expression was detected in mice administered ssAAV9, whereas efficient GFP expression was achieved throughout the entire brain using dsAAV9 (Fig. 5). Thus, systemic administration of the dsAAV9 vector appears to be an effective means of transducing the CNS in adult mice. It was demonstrated that combined injection of AAV vectors with mannitol [13, 14] or use of ultrasound-targeted microbubble destruction [15] enhances gene expression in the brain after systemic injection of AAV vectors. Therefore, to improve gene delivery in the brain, systemic administration of the dsAAV9 vector, along with these strategies, may be a powerful tool for transduction to the CNS.

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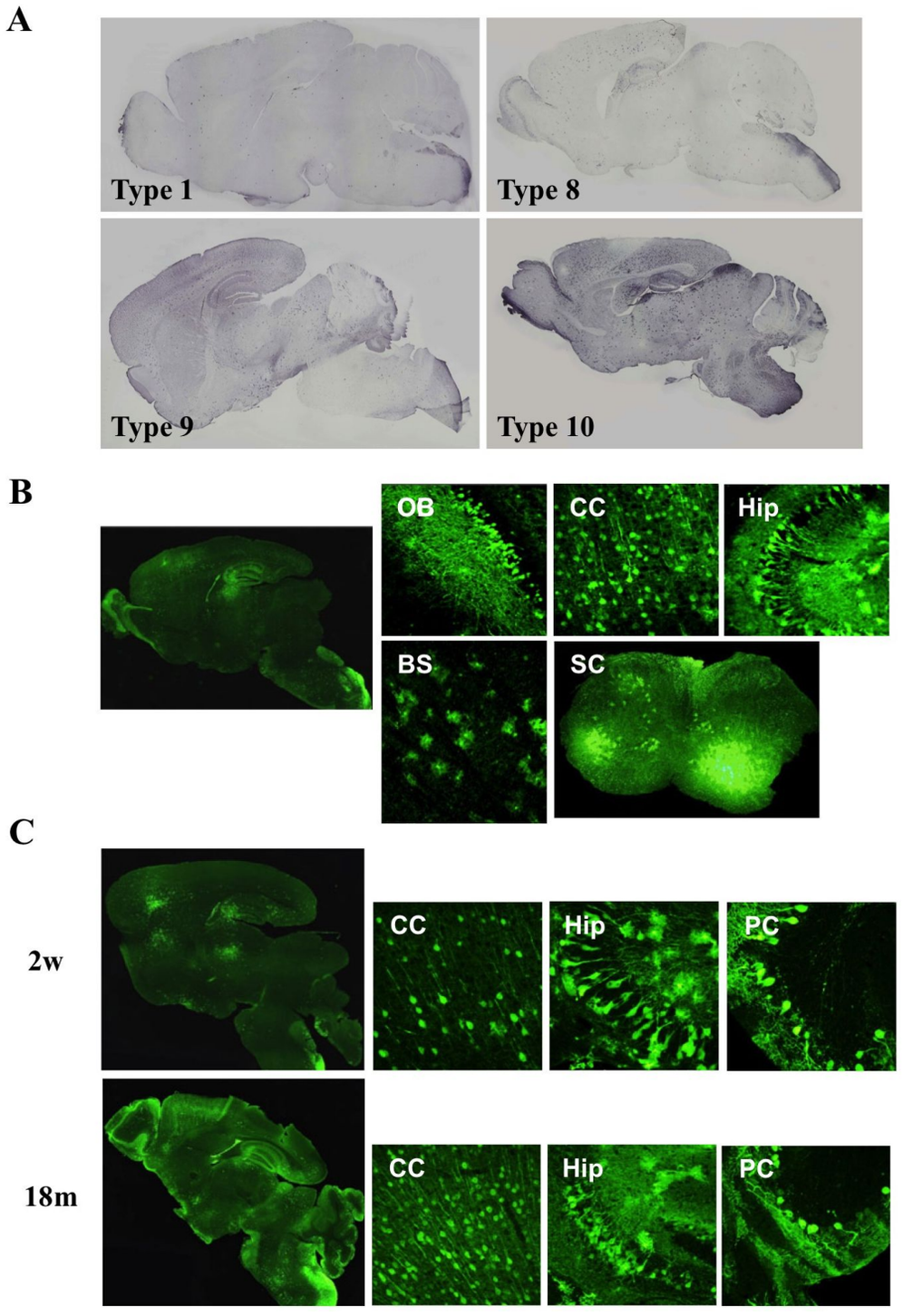

OB, olfactory bulb; CC, cerebral cortex; Hip, hippocampus; PC, Purkinje cells in the cerebellum; BS, brain stem; SC, spinal cord.

Figure 4. Direct comparison of AAV serotypes to transduce CNS by neonatal systemic injection. (A) After serotype-1, -8, -9, or -10 AAV/GFP vectors were intravenously injected into neonatal C57BL/6 mice, cerebral GFP expression was analyzed by DAB staining 4 weeks after injection. Representative brain images showing immunohistochemistry using an anti-GFP antibody after AAV9/GFP (B) and AAV8/GFP (C) injection.

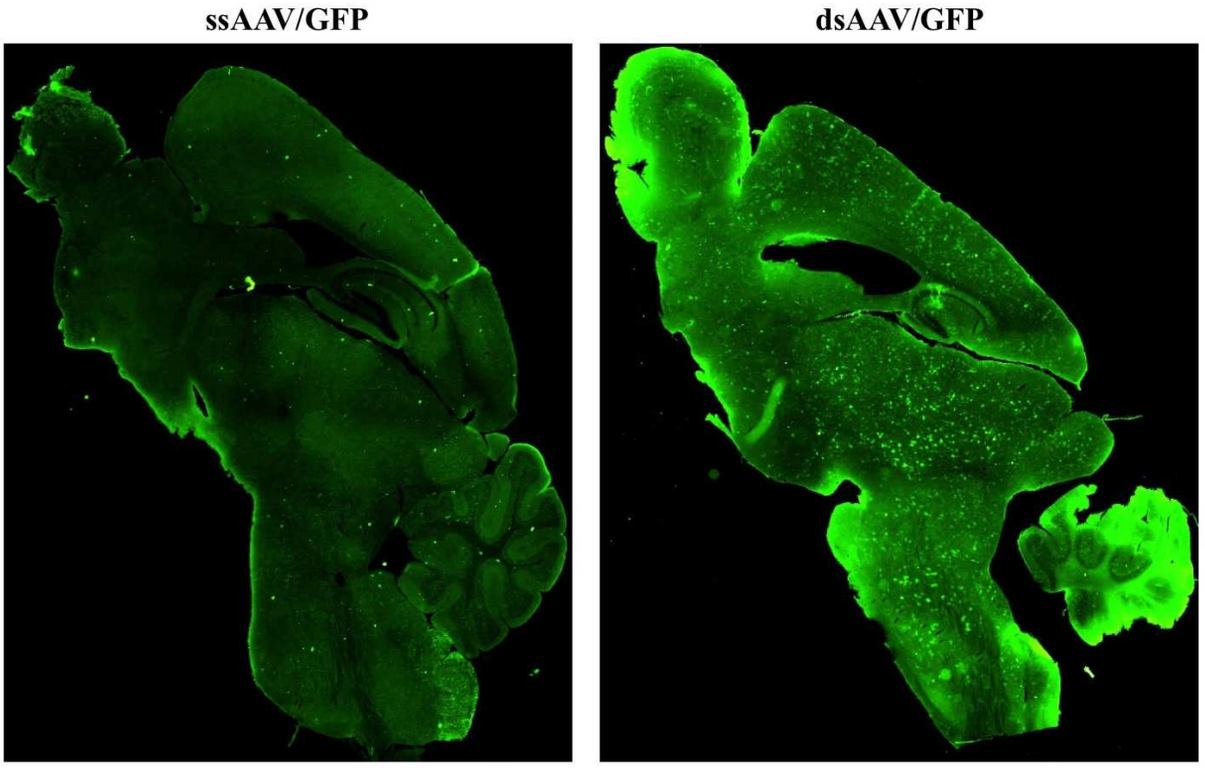

Figure 5. Immunohistochemical staining of brain sections of adult mice following systemic injection of ssAAV9/GFP or dsAAV9/GFP vectors. After 7.0 x 10^{12} vg of AAV9/GFP vectors were injected via tail veins of adult (7-week-old) mice, expression of GFP was analyzed using fluorescent microscopy at 5 weeks post administration.

4.2. Intracerebroventricular and intrathecal injection of AAV vectors

Another strategy for achieving global gene transfer into the CNS through systemic administration is vector delivery into the cerebrospinal fluid (CSF). There are two approaches to delivering an AAV vector into the CSF: intracerebroventricular injection and intrathecal injection. To evaluate the feasibility of intracerebroventricular injection, AAV1/GFP vectors were injected into the right lateral ventricle. Following the injection, GFP expression was broadly distributed in the choroid plexus and ependymal cells throughout the cerebral ventricles (Fig. 6A). Coronal brain sections revealed widespread diffusion of AAV1 from the injection site to the contralateral, anterior lateral and third ventricles, as well as the fourth ventricles via the cerebral aqueduct [16]. GFP expression was mainly confined to the choroid plexus and ependymal cells, with little or no detection of GFP in the brain parenchyma or spinal cord. Similarly, when we administered the AAV1/GFP vector intrathecally, GFP expression was broadly distributed throughout the brain (Fig. 6B). In addition, large numbers of nerve fibers in the dorsal spinal cord and the neuronal cell bodies in the dorsal root ganglia were also efficiently transduced [17]. Thus, it can be concluded that both intracerebroventricular and intrathecal injection of AAV vectors are useful for transduction of the CNS, especially if one wants to also transduce the peripheral nervous system.

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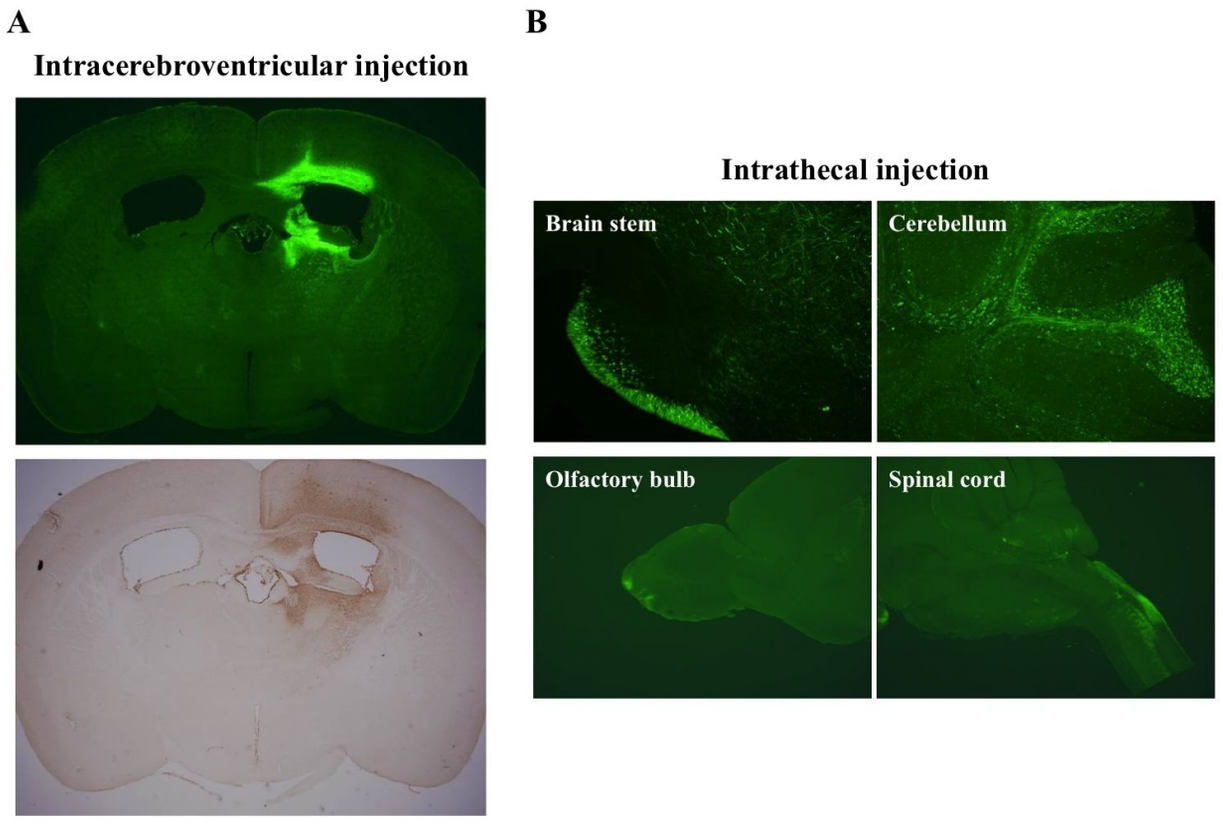

Figure 6. Expression of GFP in the brain after intracerebroventricular or intrathecal injection of AAV vectors. (A) After injection of AAV1/GFP vectors into the right lateral ventricle, GFP expression in the brain was analyzed by fluorescence microscopy (upper panel) or immunostaining with DAB staining (lower panel). (B) AAV1/GFP vectors were injected into the posterior cistern of 8-week-old mice and the brains were examined 8 weeks after injection. GFP expression was monitored by fluorescence microscopy.

5. Application of AAV vectors to CNS disorders

As summarized above, AAV vectors are an effective means of delivering genes into the CNS, thanks to their ability to transduce post-mitotic neurons and mediate efficient and stable transduction. Indeed, the utility of directly delivered AAVs has been demonstrated in numerous preclinical studies, and they are currently being used in clinical trials of treatments for Alzheimer's disease [18, 19], Parkinson's disease [20, 21], Canavan's disease [22], and Batten's disease [23, 24], among others. We also evaluated the utility of brain directed [25], intracerebroventricular [16], intrathecal [17], and intravenous neonatal administration [26] of AAV vectors for the treatment of metachromatic leukodystrophy (MLD), an inherited lysosomal storage disease with severe neurological symptoms. When we injected AAV9 vectors expressing human arylsulfatase A (AAV9/ASA) into the jugular vein of newborn MLD model mice, efficient ASA expression was detected throughout the entire brain (Fig. 7A) and peripheral nervous system (Fig. 7C), suppressing the accumulation of sulfatides in both CNS (Fig. 7B) and PNS (Fig. 7D). Moreover, the treated mice showed a greater ability to traverse

narrow balance beams than untreated mice [26]. These data clearly demonstrate that MLD model mice can be effectively treated through systemic administration of AAV9/ASA vector to neonates. Thus, neonatal gene therapy is one approach with the potential to overcome the limitation imposed by the BBB on treating genetic disorders of the CNS. Other advantages of systemic gene transfer to neonates with genetic disease over treatment of adults are as follows: (1) because the immune system is immature, recipients are immunologically tolerant of the vector; (2) it may prevent early onset of genetic diseases; (3) neonates can be effectively treated with a smaller amount of vectors than adults; and (4) nearly all organs are efficiently transduced. Systemic neonatal gene therapy thus appears to be a promising method for treating systemic genetic diseases with neurological symptoms.

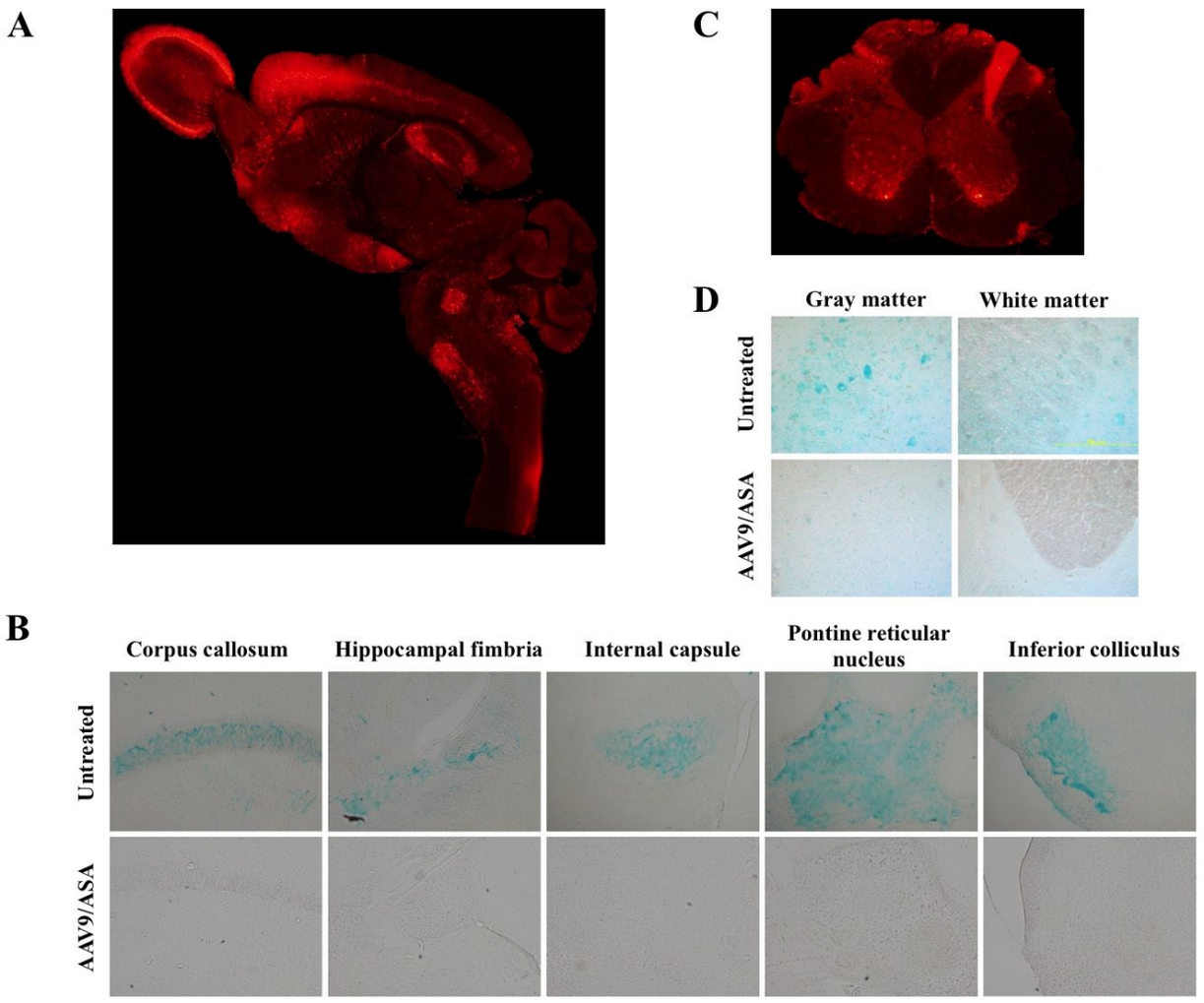

Figure 7. Correction of sulfatide storage by neonatal systemic injection of AAV9/ASA vectors. After injection of AAV9/ASA into the jugular vein of newborn MLD mice, ASA expression in the brain (A) and spinal cord (C) was analyzed by immunohistochemistry using an anti-ASA antibody at 15 months after injection. Correction of sulfatide storage in the brain (B) and spinal cord (D) was analyzed by alcian blue staining.

6. Summary and future developments

In summary, AAV vectors are a promising tool to transduce both the CNS and the spinal cord. Following a single systemic injection, AAV vectors cross the BBB and mediate widespread gene transduction throughout the brain, including the cerebral cortex, cerebellum, olfactory bulb, and brain stem. Recently, to obtain more efficient transduction, a new AAV vector serotype [27] or tyrosine mutant capsid [2] was developed. In addition, Rafi et al. succeeded in treating a mouse model of Krabbe disease (twitcher mice) by administering a combination of intracerebroventricular, intracerebellar, and intravenous injections of AAV vectors to neonates [28]. This suggests that administration of AAV vectors via several routes could prove highly useful for efficient and long-term overexpression or downregulation of genes throughout the CNS and spinal cord and could be a useful means of treating genetic neurological diseases.

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Author details

Koichi Miyake*, Noriko Miyake and Takashi Shimada

*Address all correspondence to: kmiyake@nms.ac.jp

Department of Biochemistry and Molecular Biology, Division of Gene Therapy, Research Center for Advanced Medical Technology, Nippon Medical School, Japan

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