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Toward a gene therapy for neurological and somatic MPSIIIA

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Mucopolysaccharidosis Type IIIA (MPSIIIA) represents an unmet medical need. MPSIIIA shares with many other lysosomal storage disorders (LSD) the characteristic of being a severe neurodegenerative disease accompanied by mild somatic involvement. Thus, the main target organ for the development of new treatments is the central nervous system (CNS), but overall clinical efficacy would be greatly enhanced by simultaneous correction of peripheral disease. We have recently developed a novel treatment for MPSIIIA based on the delivery to the cerebrospinal fluid of serotype 9 adeno-associated virus (AAV9)-derived vectors. This gene therapy strategy corrected both CNS and somatic pathology in animal models through widespread transduction of CNS, peripheral nervous system (PNS), and liver. The work set the grounds for the clinical translation of the approach to treat MPSIIIA in humans. Here we discuss some important considerations that further support the applicability of this treatment to MPSIIIA and other LSD with CNS and somatic involvement.

Keywords: MPSIIIA, LSD, CNS gene therapy, CSF, AAV

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Introduction

Mucopolysaccharidosis Type IIIA (MPSIIIA), or Sanfilippo Syndrome Type IIIA, is an autosomic recessive neurodegenerative metabolic disease caused by the deficiency of sulfamidase (SGSH), a sulfatase involved in the stepwise degradation of the glycosaminoglycan (GAG) heparan sulfate (HS).[1] Sulfamidase is active within the lysosomes of cells, hence the lack of activity of this enzyme causes the progressive accumulation of undegraded forms of HS within these organelles and, subsequently, lysosomal and cellular dysfunction. As the genetic defect affects all cells of the organism, a certain degree of lysosomal pathology occurs in all tissues of the body, but the disease most serious clinical manifestation is progressive global neurodegeneration, which is accompanied by a mild somatic pathology.[1,2] The disease manifests around 1–4 y of age, generally with delayed psychomotor development and behavioral problems, which are followed by a rapid, progressive loss of cognitive and motor skills.[1,2] Non-neurological alterations include hepato- and splenomegaly, frequent diarrhea, recurrent ear, nose and throat infections, and facial dysmorphisms.[1,2] Neurological and non-neurological disease worsen with age and lead to death of affected individuals during late adolescence,[1,2] although in certain cases slower progression and extended lifespan have been described.[3]

As for most of these LSD diseases, there is currently no approved treatment for MPSIIIA, although a few therapeutic strategies are currently under clinical investigation. Finding a cure for diseases that affect diffuse areas of the CNS, such as MPSIIIA, is challenging, mostly due to the presence of the blood brain barrier (BBB) that limits the entry to the CNS of systemically administered drugs.[4] One important concept to keep in mind when developing a therapy for a LSD is that soluble lysosomal enzymes present in the extracellular compartment are taken up by mannose-6-phospate receptor (M6PR)-mediated endocytosis into affected cells.[4] Based on this principle, the enzyme produced by one healthy cell can cross-correct neighboring cells carrying the disease.

This principle explains why for several LSD bone marrow transplantation represents a therapeutic option; it also explains why the correction of the genetic defect in all cells of an organ is not a requirement for gene therapy-based strategies, as few corrected cells will, in principle, secrete sufficient amounts of enzyme that will then become available to neighboring cells.

Among the therapies tested in MPS IIIA, one is the delivery of the therapeutic agent directly to the CNS by periodic administrations of recombinant protein to the cerebrospinal fluid (CSF) through a permanently implanted intrathecal delivery device (NCT01155778 and NCT01299727, clinicaltrials.gov). While this approach is technically feasible, it is highly invasive, and the presence of a permanent implant may carry risks of complications such as infections. Results from this approach are yet to be published. A second approach tested is a gene transfer strategy in which adeno-associated virus (AAV)-derived vectors of serotype rh10 (AAVrh10) encoding for the sulfamidase and sulfatase-modifying 1 (SUMF1) transgenes are delivered through multiple direct injections to the brain parenchyma (NCT01474343, clinicaltrials.gov). This is also a very invasive approach that fails to transduce the entire CNS and brain stem, as shown in preclinical studies for Batten disease.[5] Long-term follow up data from the recently concluded MPS IIIA trial will help addressing this important point.

Intra-CSF Delivery of AAV9 Vectors as a New Therapeutic Approach

We recently demonstrated in animal models the safety and feasibility of correcting whole-body MPSIIIA disease with a novel gene therapy strategy based on the delivery to the CSF of AAV9 vectors carrying the sulfamidase gene, which leads to widespread transduction of the encephalon, spinal cord and liver (**Fig. 1**).[6] Although other authors had found vectors in peripheral organs following delivery of AAV vectors to the CNS,[7,8] an unexpected finding of our study was that the amount of vector that reached the circulation after CSF delivery was sufficient to mediate correction of MPSIIIA somatic disease. When AAV9 vectors encoding sulfamidase were delivered through cisterna magna to MPSIIIA mice, an increase in sulfamidase activity was detected throughout the brain and in serum, being the liver the most important source of circulating enzyme (**Fig. 1**). This restoration of enzymatic activity led to correction of GAG accumulation and lysosomal pathology in brain and peripheral organs, normalization of behavioral deficits and prolonged (> 24 mo) survival of treated animals.[6] Thus, our study was the first to report whole-body correction of a lysosomal storage disease following CNS-directed gene therapy. Importantly, the pattern of distribution of the vector, which we believe to result from the broad tropism of AAV9[9,10] combined with CSF delivery, was confirmed in large animals models using two different routes of entry to the CSF (intracisternal and intracerebroventricular vector administration). Moreover, we demonstrated in dogs that our approach could lead to the secretion of AAV-derived sulfamidase to the CSF, where it remained at high levels, in the absence of any signs of inflammation or toxicity, for the 3-mo follow-up period (with observation ongoing).

Our study provides the grounds for the clinical translation of intra-CSF delivery of AAV9-sulfamidase vectors for the treatment of MPSIIIA. By providing the sulfamidase gene to the affected cells, instead of administering the recombinant protein, the therapeutic benefit deriving from our proposed approach is expected to be long-lived, likely requiring a single product administration. AAV vectors have emerged as promising in vivo gene transfer tools, showing long-term production of therapeutic proteins in animal models and in humans,[11,12] with data from human trials accounting for therapeutic transgene expression up to 10 y after a single vector administration.[13] On the other hand, the delivery of AAV vectors to the CSF ensures widespread, even, distribution of transduced cells throughout the brain and other important CNS structures with a minimally invasive procedure.

Extensive biodistribution studies in dogs showed that vector genomes could be detected in > 35 random tissue samples from both hemispheres of the encephalon, independent of the route used to deliver the vector to the CSF. Moreover, gene copy numbers ranged from 0.1 to 17 vector genomes (vg) per cell, with an average value around 2.5, evidencing a very homogenous distribution of the vector, which could be detected in deep structures such as the pons, medulla oblongata, and cerebellum.[6] In contrast, when AAVs are administered to the brain parenchyma, and due to the limited diffusion of vectors from the point of injection,[5,14] the profile of vector distribution is uneven, with extremely high vector genomes at the point of injection but quickly decreasing with distance.[7,15] Furthermore, as there is a limit to the number of injections and to the locations at which these injections can be safely performed, intraparenchymal delivery fails to transduce deep CNS structures.[14] The impossibility to target the cerebellum and brainstem has been accounted a likely culprit for the limited therapeutic efficacy observed in the Canavan disease trial.[16]

Vector Dose and Volume of the Target Organ

The extrapolation, by brain volume, of the total dose used in our mouse and dog studies to children results in a therapeutic dose of approximately 1.4×10^{14} vg, which is considerably higher than the doses previously used in CNS-directed, in vivo gene therapy clinical trials in adult and pediatric populations.[16-20] Assuming the brain as the only organ of distribution of the vector, and a mean brain volume of 1260 ml,[21] our proposed clinical dose is 1.1×10^{11} vg/ml organ. This value is a slight overestimation, as it does not take into account the overall distribution of the vector, i.e., the vector genomes that do not transduce the brain but transduce the spinal cord, dorsal root ganglia and liver. A similar analysis of the relationship between the total dose of vector administered and the volume of the target organ reveals that our proposed clinical dose is not outside the range of the doses used in human studies for which there is clear evidence of efficacy with excellent safety record. For example, in a randomized, double-blind, sham-surgery controlled Phase II clinical

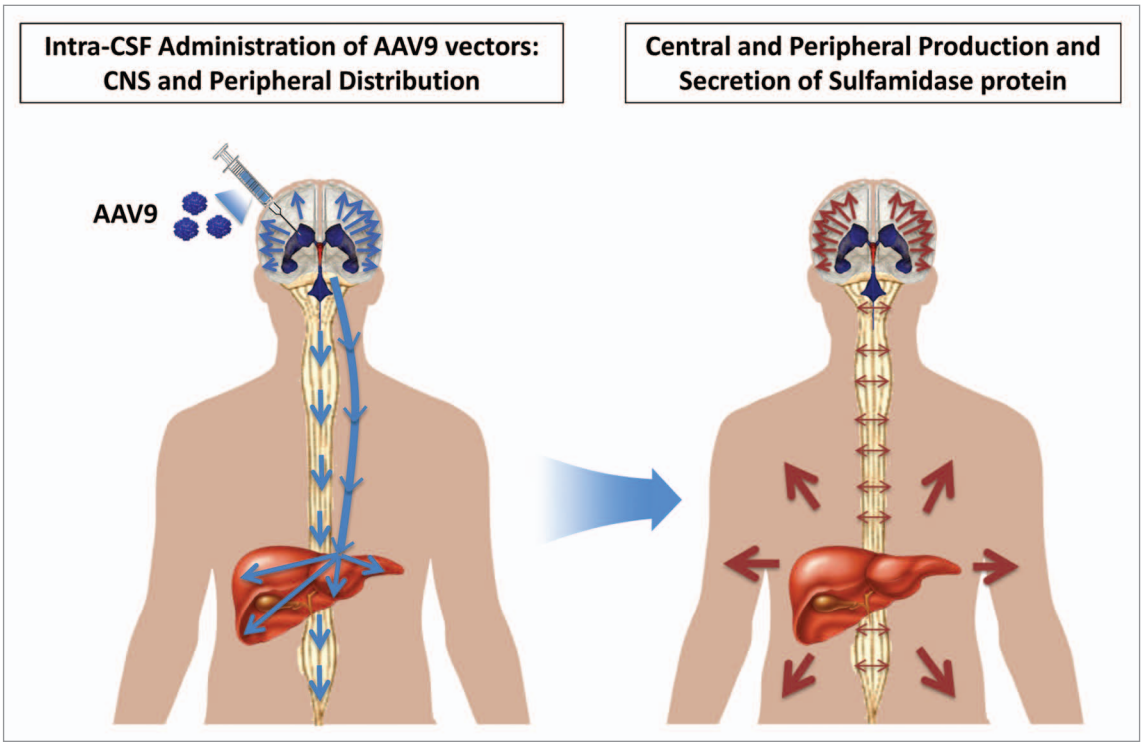

Figure 1. Schematic representation of vector and transgene product distribution following intra-CSF delivery of AAV9 vectors. The delivery of AAV9 vectors to the CSF through unilateral administration to the lateral ventricle leads to widespread distribution of vector particles throughout the brain and spinal cord. In addition, some vector reaches the circulation, leading to the transduction of the liver (left). As a result of this profile of vector distribution, sulfamidase activity increases throughout the CNS, in the CSF and in serum, being the liver the most important source of circulating enzyme (right).

trial for Parkinson disease in which a clear improvement in the primary endpoint was observed, AAV2 vectors encoding the glutamic acid decarboxylase (GAD) gene were delivered to each subthalamic nucleus at a dose of ~3.5 × 10^{10} vg.[19,22] In Parkinson patients, the volume of the subthalamic nucleus has been estimated to be 0.13 ± 0.01ml,[23] hence the dose per ml of target organ used in this trial was 2.7 × 10^{11} vg/ml. Similarly, a small amount of vector (1.5 × 10^{11} vg) has been delivered to the subretinal space for the treatment of Leber's congenital amaurosis due to RPE65 deficiency with an excellent safety profile and very promising outcome.[24,25] No data are available on the volume of the subretinal space, but if the volume of the whole eye were to be used for the calculation, being 6.5 ml for an adult human,[26] then the dose per ml used in this clinical study was 2.3 × 10^{10} vg/ml. As mentioned before, the intra-CSF delivery of AAV9 vectors results in uniform distribution of vector across the brain,[6] which in theory, together with the immunological privilege of the CNS,[27] would prevent the development of unwanted immune responses. Future studies will provide evidence of the safety of this gene transfer approach in humans.

Amenability for Clinical Development of the ICV Delivery Procedure

The route we chose to use to deliver vectors in our strategy maximizes correction in the disease most important target organ, the brain, but also provides systemic therapeutic benefit through transduction of a percentage of hepatocytes, which is sufficient to produce and secrete enzyme to the circulation from where it becomes available to all somatic organs.[6] Most importantly, we managed to achieve widespread transduction of the brain, the brainstem, the medulla oblongata, the cerebellum, the whole spinal cord—up to the cauda equina—and even all dorsal root ganglia of the peripheral nervous system with a surgical procedure than is minimally invasive.[6] Although in proof-of-concept studies vectors were delivered through intracisternal injection to mice and dogs, this route of administration to the CSF is not common in clinical pediatric practice, due to the relatively smaller size of the cisterna in humans compared with animals and its proximity to vital centers. Thinking of the most optimal approach for clinical translation, and with the aim of increasing the safety of the delivery procedure, we explored the possibility of administering the vectors through intracerebroventricular (ICV) injection, a technique commonly used in pediatric neurosurgery.[28] Although ICV access does require the unilateral trepanation of the skull (only one burr hole is needed to administer the vector through this route), the trajectory to reach the ventricle is well defined and goes through "mute" areas of the brain.[28] The placement of a catheter in the ventricle, or ventriculostomy, is used for conditions such as the management of hydrocephalus, or for the administration of pharmaceuticals like oncology drugs, antibiotics or antifungal agents, or medications for the treatment of severe chronic pain, spasticity and dystonia,[29] with more

than 40 000 procedures performed per year in the US alone.[30] Using a surgical procedure that has been in place for many years provides a great safety record and a very well defined list of potential delivery-associated adverse events. It also simplifies clinical translation, as the technique is known to pediatric neurosurgeons worldwide and would not require specific training. One additional advantage of ICV administration is that by delivering the vector to the CSF fluid, rather than to the parenchyma, a relatively large volume of vector can be supplied within a brief period of time, thus shortening the duration of the surgery and providing flexibility in terms of vector concentration and formulation. In contrast with ICV vector delivery, administration of vector through multiple burr holes[16,17] required the design of purpose-specific devices to deliver the vector within a practical time frame and the implementation of new neurosurgical techniques.[14]

Impact of Preexisting Immunity against AAV on Intra-CSF Delivery of Vectors

AAVs are common, non-pathogenic viruses and the great majority of the adult population has anti-AAV antibodies in serum, which can greatly limit the efficacy of in vivo gene transfer upon systemic administration.[31,32] The prevalence and magnitude of seropositivity, however, varies with the AAV serotype.[33] While ~60% of the adult population has anti-AAV2 neutralizing antibodies (NAbs) at high titers, only 30% of healthy individuals have detectable anti-AAV9 antibodies, and at much lower titers compared with AAV2.[33] Other serotypes with low seropositivity prevalence include AAV5 and AAV8.[33] Nonetheless, independent of the serotype, there seems to be a pattern of seroconversion with age: children under 1 y are seronegative or seropositive at very low titers, then titers progressively increase up to around 5–6 y of age, reflecting the increase of socialization as children are scholarized, which favors natural infection by wild-type AAVs.[34,35] This point is of utmost importance for the treatment of pediatric diseases with in vivo AAV gene therapy protocols, because a great percentage of the target population would be naïve to the vector.

In a small cohort of healthy and MPSIIIA-affected children we observed the same tendencies discussed above: titers against AAV2 were generally higher than those against AAV9 and they tended to increase with age in both cases, being the majority of children younger than 6 y seronegative.[6] Interestingly, in the same cohort we measured the levels of NAbs in matched CSF samples. In all cases, and independent of the serotype or the titer in serum, CSF titers were extremely low or undetectable.[6] This finding suggests that even in diseased individuals the BBB integrity is maintained, or at least the asymmetric distribution of immunoglobulins between serum and CSF is retained.

To further explore how pre-existing immunity could impact the therapeutic efficacy of intra-CSF delivery of AAV9 vectors, we designed a very astringent study in which healthy dogs were pre-immunized by systemic administration of non-coding AAV9. A month post-immunization, dogs had high titers of anti-AAV9 in serum (1:100–1:1000). In contrast, titers in CSF were very low (1:1–1:3). At this point, dogs received an intra-CSF administration of AAV9 vectors encoding a marker gene. Even after this direct CNS administration of vectors, the titers of NAbs in the CSF remained low, while the titers in serum increased significantly, as expected. The systematic evaluation of transduction revealed that although all somatic efficacy was lost due to the high levels of serum NAbs at the time of vector administration, most of the transduction in the CNS remained, with 70–90% of encephalon and spinal cord samples testing positive for the presence of vector genome and transgene expression.[6] Although further studies are required to verify how the presence of pre-treatment low NAb titers could impact, for example, the levels of sulfamidase secreted to the CSF, these results suggest that treatment through intra-CSF delivery is still feasible in children with pre-existing immunity. Despite the lack of somatic efficacy, the minimal impact that pre-existing humoral immunity had on intra-CSF delivery of AAV9 vectors represents a clear benefit over other approaches that take advantage of AAV9's ability to cross the BBB after systemic intravenous delivery.[36,37]

Reversibility in Chronic Storage Diseases

One important aspect of the experimental design of our study is that we chose to use MPSIIIA animals that were 2 mo old at the time of vector administration. We and others had previously demonstrated that animals already have established disease at this age, as indicated by the accumulation of GAGs in the liver[38] and brain[36,38,39] and the presence of neuroinflammation.[36] Moreover, the degree of neuropathy is such at 2 mo of age that animals show clear behavioral abnormalities in the open field test,[40] the same test by which we demonstrated normalization of behavior in treated MPSIIIA mice four months after intra-CSF AAV9-sulfamidase treatment.[6] The treatment-mediated correction of behavioral deficits was accompanied by the disappearance of GAG accumulation, lysosomal pathology and neuroinflammation in the brain, all of which were at the levels observed in healthy mice, and by normal lifespan, suggesting the treatment not only prevented but also reverted established MPSIIIA disease. Moreover, in an unpublished study we observed that 4 mo after systemic delivery of AAV9-sulfamidase vectors to old (~6 mo of age) MPSIIIA mice, GAG content was completely normalized in the brain and liver of treated animals, the size of the brain lysosomal compartment was also normal and neuroinflammation disappeared (Bosch et al., unpublished data). Given that in this study animals were sacrificed at an age (10 mo) at which untreated MPSIIIA mice already begin to die,[36,38] the observations give further support to the conclusion that lysosomal pathology is reversible, although further studies are required to determine if there is a point of no return after which functional recovery is no longer achievable despite the clearance of lysosomal storage. In agreement with our results, reversal of established disease following gene therapy has been documented in the context of other LSD, such as metachromatic adrenoleukodistrophy.[41] These observations have important implications for the future

clinical application of the treatment. Rare genetic diseases have large variability in clinical manifestation, and the progression of signs and symptoms in patients suffering from the same disease can vary significantly from one patient to another. In other words, although the clinical course is comparable, the age at presentation of signs and symptoms will be variable between patients. Patients with a severe clinical form, which is the most frequent case among MPSIIIA patients, are diagnosed at an early age and mental deficit and loss of functions occur also at an early age, earlier than in patients with a more attenuated phenotype.[2,3] Regardless of this variability, the majority of MPSIIIA patients are diagnosed between 1–4 y of age, once neurodegenerative disease has already manifested clinically.[1-3] Most studies, however, indicate that loss of functions occurs at around 10 y of age.[1,2,4] If loss of function were to be considered a point of no return, then there is a window of opportunity for successful treatment of several years after disease diagnose.

Concluding Remarks

The in vivo delivery to the CSF of AAV9 vectors encoding a therapeutic gene holds great potential for the treatment of MPSIIIA and other LSD with widespread neurological involvment, in which the treatment of the CNS disease is a priority. This gene transfer strategy maximizes the distribution of the product in the CNS while minimizing delivery-associated risks. Additionally, the approach could provide clinical benefit to the somatic disease that often accompanies neurodegeneration in several LSD, which would become more relevant as patients live longer due to improved neurological outcome. Further studies to determine the safety, tolerability and potential clinical efficacy of this therapeutic strategy are warranted.

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.

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