Comparison of Endovascular and Intraventricular Gene Therapy With Adeno-Associated Virus–α-L-Iduronidase for Hurler Disease

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BACKGROUND: Hurler disease (mucopolysaccharidosis type I [MPS-I]) is an inherited metabolic disorder characterized by deficiency of the lysosomal enzyme α-L-iduronidase (IDUA). Currently, the only therapies for MPS-I, enzyme replacement and hematopoietic stem cell transplantation, are generally ineffective for central nervous system manifestations.

OBJECTIVE: To test whether brain-targeted gene therapy with recombinant adeno-associated virus (rAAV5)-IDUA vectors in an MPS-I transgenic mouse model would reverse the pathological hallmarks.

METHODS: Gene therapy approaches were compared using intraventricular or endovascular delivery with a marker (rAAV5-green fluorescent protein) or therapeutic (rAAV5-IDUA) vector. To improve the efficiency of brain delivery, we tested different applications of hyperosmolar mannitol to disrupt the blood-brain barrier or ependymal-brain interface.

RESULTS: Intraventricular delivery of 1×10^{11} viral particles of rAAV5-IDUA with systemic 5 g/kg mannitol co-administration resulted in IDUA expression throughout the brain, with global enzyme activity >200% of the baseline level in age-matched, wild-type mice. Endovascular delivery of 1×10^{12} viral particles of rAAV5-IDUA to the carotid artery with 29.1% mannitol blood-brain barrier disruption resulted in mainly ipsilateral brain IDUA expression and ipsilateral brain enzyme activity 42% of that in wild-type mice. Quantitative assays for glycosaminoglycans showed a significant decrease in both hemispheres after intraventricular delivery and in the ipsilateral hemisphere after endovascular delivery compared with untreated MPS-I mice. Immunohistochemistry for ganglioside GM3, another disease marker, showed reversal of neuronal inclusions in areas with IDUA co-expression in both delivery methods.

CONCLUSION: Physiologically relevant biochemical correction is possible with neurosurgical or endovascular gene therapy approaches for MPS-I. Intraventricular or endovascular delivery of rAAV5-IDUA was effective in reversing brain pathology, but in the latter method, effects were limited to the ipsilateral hemisphere.

KEY WORDS: AAV, Blood-brain barrier, Gene therapy, Hurler disease, Mannitol, Mucopolysaccharidosis

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ABBREVIATIONS: ECA, external carotid artery; **GAG,** glycosaminoglycans; **GFP,** green fluorescent protein; **g.p.,** genomic particles; **IA,** intra-arterial; **ICA,** internal carotid artery; **ICV,** intracerebral ventricular; **IDUA,** α-L-iduronidase; **MPS-I,** mucopolysaccharidosis type I; **rAAV,** recombinant adeno-associated virus; **scAAV,** self-complimentary adeno-associated virus

Hurler disease or mucopolysaccharidosis type I (MPS-I) is an autosomal-recessive lysosomal storage disease caused by a defect in α-L-iduronidase (IDUA).[1,2] It is the most common of the mucopolysaccharidoses, with an incidence of 1 per 75 000 live births.[3] Deficiency of the IDUA enzyme leads to a pathological accumulation of glycosaminoglycans (GAG) in the brain and other organs. Typical central nervous system (CNS) features are severe mental retardation, leptomeningeal fibrosis with

obstructive hydrocephalus, arachnoid cysts, and sensorineural deafness. Although systemic features of MPS-I can be treated with enzyme replacement therapy or hematopoietic stem cell transplantation, there is currently no accepted treatment for the CNS features. A caveat is that intervention in the neonatal period with bone marrow or cord blood transplantation has shown limited improvement in cognitive performance, likely through cross-correction by stem cells that migrate to the perivascular space.[4,5] However, even in the best-case scenario, the existing treatment options do not reverse abnormal cognitive development, are associated with numerous long-term complications, and are prohibitively expensive for many patients.

The pathological hallmark of MPS-I in the brain is the accumulation of GAG inside neurons, where they are increased up to 5-fold, as well as in mesodermally derived tissues of the perivascular space, leptomeninges, and choroid plexus, where they are often increased 10-fold and form so-called clear-cell periadventitial inclusions.[6] As a result of pathological accumulation of GAG, there is a secondary perturbation of β-galactosidase (originally thought to be the main defect in Hurler disease),[7] and consequently, the neuronal inclusions in Hurler disease are made up primarily of gangliosides (GM2, GM3) which are not usually present in substantial amounts in normal brain.[8,9] Another downstream enzyme, β-hexosaminidase (defective in Tay-Sachs disease), which breaks down GM2, is upregulated in MPS-I, and normalization of this enzyme was used as a secondary outcome measure in canine gene therapy studies for Hurler disease.[10]

Our inability to achieve high-level neuronal gene expression throughout the large volume of the human brain is currently an impediment to more effective gene therapy for many neurodegenerative diseases. The purpose of this study was to test the feasibility of minimally invasive viral vector-based CNS gene therapy in Hurler disease and, more specifically, to compare endovascular with intracerebral ventricular (ICV) delivery. Recombinant adeno-associated viral vector (rAAV) was chosen because it is nonpathogenic and is able to express genes in the CNS at very high levels.[11] It exists in more than 10 serotypes, most of which specifically transduce neurons when injected intraparenchymally, but some are capable of transducing astrocytes, microglia, ependyma, and other cells to various degrees. To maximize the unit dose per animal, we used a transgenic mouse model[12] rather than one of the larger animal models,[13,14] with an aggressive endovascular dosing regimen of 1 trillion genomic particles (g.p.) per mouse. We chose rAAV5 vector, which has an advantage of high-level neuronal expression and cellular internalization by the platelet-derived growth factor receptor,[15] which also targets ependymal, choroid plexus, and perivascular cells affected in Hurler disease. Another theoretical benefit of rAAV5 is that neutralizing antibody titers are the lowest among AAV serotypes.[16]

Endovascular gene therapy offers widespread access to the brain but generally requires blood-brain barrier disruption, and until recently, results in animal models were disappointing. Rapoport and colleagues[17,18] were the first to show reversible osmotic opening of the blood-brain barrier, and their technique of hyperosmolar disruption remains in widespread use. This method was adapted for clinical use in intra-arterial (IA) chemotherapy for brain tumors[19] but has never been used clinically for gene therapy. Endovascular techniques have been used in rats[20-22] or mice[23] to deliver viral vectors to the brain, but gene expression was significantly less than with intraparenchymal delivery, and the microsurgical techniques are demanding. As a result, this approach was abandoned by the majority of gene therapists, even as microcatheter techniques and vector technology advanced.

ICV gene therapy is appealing because the ependymal and meningeal surface area is large and may be particularly suited to Hurler disease, which involves these structures. The original clinical application of ICV gene therapy in 1996 used a nonviral vector with Ommaya reservoirs in patients with Canavan disease together with intravenous mannitol.[24] The benefit of mannitol for this route of delivery is the greater bulk flow through the interstitial space and increased permeability of the ependyma, rather than effects on the blood-brain barrier. Systemic mannitol was shown in animal models to increase spread of vectors with ICV or intraparenchymal delivery.[24-26] However, as a result of perceived limitations of the ICV route with viral vectors, an intraparenchymal approach with multiple rAAV2 injections and systemic mannitol was subsequently adopted for human use.[27,28] At this time, the ICV approach deserves a closer look with new vector technology and techniques to optimize transependymal flow.

Our study used rAAV5 vectors in adult animals to deliver foreign genes to the brain via IA or ICV delivery, with mannitol treatment used in both cases. In the first case, mannitol is administered directly to arterial vessels to transiently disrupt the endothelial tight junctions and to allow penetration of vector, whereas in the latter case, mannitol is used to improve bulk flow of rAAV5 through ependyma and interstitial space. We initially tested dose-response and biodistribution using rAAV5–green fluorescent protein (GFP) and then assessed the tolerability and efficacy of a very high unit dose (5×10^{13} viral particles per kg) of the therapeutic vector rAAV5-IDUA. With IA delivery, blood-brain barrier disruption was performed with 25% or 29.1% mannitol, and with ICV delivery, we used 25% mannitol injected systemically or directly into the cerebral ventricle. Our goal with ICV delivery was not merely to assess the use of mannitol, because this is already known to increase vector spread,[24,29] but rather to compare the endovascular and ICV delivery routes using the best available techniques. Using either delivery paradigm, we significantly reversed brain pathology in animals with a Hurler phenotype.

PATIENTS AND METHODS

Construction of AAV5-IDUA and AAV5-GFP Vectors

The human IDUA sequence[30] and plasmid cloning techniques were previously described.[31] The plasmid pTR-MCI contains the human IDUA expression cassette with CBA promoter and other elements, flanked by AAV2 ITR sequences. This plasmid was amplified and used to prepare pseudotyped rAAV2/5 viral vector at the University of Florida Powell Vector Core. After column purification, genomic titers were

verified by dot blot. Before use in vivo, the rAAV5-IDUA vector was used to transduce HEK293 cells that were verified for expression of IDUA by Western blot. The plasmid pTR-UF11 contains a humanized GFP gene with CBA promoter flanked by AAV2 ITR sequences and was similarly used to generate rAAV5-GFP.

MPS-I Mice: Group Allocation and Surgical Procedure

Homozygous MPS-I mice were obtained from a breeding colony at the University of Minnesota, with founder animals donated by Dr Elizabeth Neufeld. Animals were handled in accordance with Institutional Animal Care and Use Committee requirements (protocol 0711A21061). IDUA-null mice were verified by genotyping from tail clippings, as previously described.[32] Mice were treated at 8 to 11 weeks of age, the minimum age at which the surgical protocol was feasible. Treatment at this age permitted emergence of a mild MPS-I phenotype before biochemical correction. Mice typically become symptomatic along with brain inclusions at around 4-8 weeks.[12] Twenty-one animals were used for preliminary rAAV5 dosing experiments, and 17 additional animals were used for gene transfer experiments. Before the procedure, each mouse was anesthetized with 100 mg/kg ketamine and16 mg/kg xylazine. For ICV delivery, the animal was secured in a Kopf stereotactic frame, and the lateral ventricle was targeted with a Hamilton syringe (AP, +0.4 mm from bregma; ML, +0.8 from midline; depth, 2.4 mm) using standard surgical techniques. For preliminary experiments with rAAV5-GFP, 25.0% mannitol was administered to the ventricle in a 1:1 ratio with vector (5 μL mannitol; approximately 0.05 g/kg body weight) or through systemic intraperitoneal injection at 2 mL/100 g body weight (approximately 5 g/kg body weight), as adapted from previously published protocol.[29] Later experiments with ICV delivery of rAAV5-IDUA used systemic mannitol delivery only. For IA delivery, the animal was positioned supine on the stereotactic base in ear bars. The forelimbs were secured with rubber loops; neck fur was shaved with a clipper; and skin was prepared in sterile fashion. A 1-cm incision was made off midline, and subcutaneous tissue was divided to expose the carotid. After temporary sutures were placed for retraction, the external, common, and internal carotid arteries were identified under surgical loupes and exposed by sharp dissection. The common carotid was clamped with a temporary microaneurysm clip, followed by the internal carotid artery (ICA). The external carotid artery (ECA) was divided and mobilized downward so that it was angled toward the carotid bifurcation. A 6-0 nylon suture was tied in a loop, and the ECA was pulled through it. The ECA was opened, and a primed PE10 catheter was inserted. The ICA clamp was removed, and the tubing was advanced and secured. Blood-brain barrier disruption was performed as described below, followed 60 seconds later by rAAV5 vector injection in a total volume of 100 μL. After another 60 seconds, the tubing was removed from the ECA, and a new suture was tied in place. Hemostasis was verified before the clamp was removed from the common carotid artery. The incision was closed with 6-0 suture.

Blood-Brain Barrier Disruption

Mannitol (Sigma) was premixed in distilled water at 1.37- or 1.60-mol/L concentration (25.0% or 29.1% wt/vol) and stored at 4°C in a sealed Pyrex tissue culture bottle with magnetic stir bar. Before use, the bottle was heated inside a 90°C water bath, with the stir bar used for mechanical agitation until completely solubilized. The solution was cooled slowly to <42°C, at which point the designated volume was loaded into a plastic tuberculin syringe, attached to preprimed tubing containing heparinized saline, and injected to the ECA. Before injection, the solution was verified to be without precipitate. The volume of mannitol solution for IA blood-brain barrier disruption was 14 μL/g body weight (approximately 4 g/kg body weight) and was infused manually over 30 seconds.

Brain Harvesting

Mice were euthanized 5 weeks after gene transfer. For biochemical assays, animals underwent endocardial perfusion with ice-cold, sterile phosphate-buffered solution. Brains were removed with rongeurs and a clean spatula, placed onto an ice-cold cutting surface, and cut sagittally into ipsilateral (right) and contralateral (left) hemispheres. Samples were immediately homogenized on ice with a prechilled glass Dounce, and homogenates were clarified and flash-frozen with the same lysis/solubilization buffer used for all assays. In brief, 1000 μL ice-cold sterile phosphate-buffered solution (pH 6.8) was added to a prechilled glass Dounce with a single brain hemisphere and gently homogenized by hand for 1 minute. Next, 10 μL of 10% Triton was added (final concentration, 0.1%), and the solution was homogenized for another 2 minutes, transferred to prechilled 2.0-mL Eppendorf tubes, kept on ice for 10 minutes, vortexed for 3 seconds, and finally centrifuged at 12 000*g* at 4°C. Each hemisphere plus buffer yields approximately 750 μL, which was split equally into tubes for the following: Bradford protein assay, IDUA assay, and GAG assay. At this point, protease inhibitors (2 μg/mL leupeptin and 0.5 mmol/L phenylmethanesulfonyl fluoride) were added to tubes for the Bradford protein assay only because protease inhibitors will adversely affect other assays. Samples were flash-frozen on dry-ice slurry and stored at −80°C until use.

Immunohistochemistry

For immunohistochemical analysis, specimens were live-perfused with 4% paraformaldehyde-buffered phosphate-buffered solution, postfixed with 4% paraformaldehyde, equilibrated in sucrose gradient to 30%, flash-frozen on dry ice, and sliced into 30-μm sections on a cryotome. Specimens were prepared as previously described[32] for immunohistochemical single and double labeling with rabbit polyclonal anti-GFP antibody (Invitrogen), monoclonal mouse anti-human GM3 antibody (Seikagaku/Associates of Cape Cod, Falmouth, Massachusetts), and monoclonal goat anti-human IDUA antibody (R&D Systems, Minneapolis, Minnesota). Optimal titers for anti-human IDUA antibodies were determined in vitro using HEK293 cells that were transduced with the rAAV5-IDUA vector. Hurler human fibroblasts (Coriell Cell Repository, Camden, New Jersey) were used side by side for negative controls to assess background. Secondary antibodies were Alexa 488 or 555 (Invitrogen) matched to the respective primary. A Nikon Eclipse E600 microscope with apochromat 4×, 10×, or 20× objectives and a SPOT Idea 5.0 megapixel monochrome charge-coupled device attachment was used for image capture, with SPOT Advanced software package calibrated according to the manufacturer's recommendations. Adobe Photoshop was used for image analysis.

Quantitative GAG Assay

Blyscan colorimetric assay for sulfated GAG (Biocolor Ltd, Carrickfergus, United Kingdom) was used according to the manufacturer's recommendations but with several modifications. Because residual protein and nucleic acids may affect the sensitivity of the assay, tissue homogenates were pretreated with proteinase K (Sigma-Aldrich), which was titrated to 3 times the amount of protein assayed by Bradford assay, at 55°C on a heating block for 24 hours, followed by boiling for 10 minutes to inactivate enzymes. Nucleic acids were then removed by adding 250 U DNAse and 2.5 U RNAse (Sigma-Aldrich) with incubation at room

temperature for 24 hours followed by boiling for 10 minutes to inactivate enzymes. All samples were run in triplicate.

Quantitative Enzyme Activity Assay

Activity of IDUA, a glycosyl acid hydrolase, was calculated with respect to wild type after normalizing to protein content (activity/unit time/mg protein) with a fluorometric assay[32,33] in which enzyme activity is measured under standard conditions with respect to the conversion of 4-methyl-umbelliferyl-α-L-iduronide to 4-methylumbelliferone. The assay conditions were validated by use of serial dilutions of purified recombinant IDUA (R&D Systems) in the 25- to 800-ng range. Enzyme activity from tissue homogenates was normalized to total protein content with the Bradford protein assay (Biorad). Samples were read on a Molecular Devices SpectraMax M5 plate reader at 365-nm excitation and 450-nm emission. Vials were coded so that the person running the samples was blinded to the contents.

RESULTS

Blood-Brain Barrier Disruption With 29.1% Mannitol (1.6 mol/L) Provides Superior Results

We began by determining the appropriate dosing range for endovascular delivery. To do this, we injected rAAV5-GFP at different viral titers to examine the brain expression of a reporter gene. The low-dose (10^{10} g.p.) and high-dose (10^{12} g.p.) cases with 25.0% mannitol blood-brain barrier disruption were compared in 10 mice. The lower dose gave virtually zero brain transduction; the higher dose gave scattered neuronal transduction (Figure 1). Biodistribution experiments in additional animals confirmed that with IA delivery, optimal brain expression was obtained with at least 10^{12} g.p. rAAV5-GFP in conjunction with 29.1% mannitol blood-brain barrier disruption (Figure 2). Conversely, 25.0% mannitol blood-brain barrier disruption resulted in very low levels of GFP expression in the brain even with high-dose 10^{12} g.p. Consistent with prior literature, rAAV5-GFP administered endovascularly without blood-brain barrier disruption or with intraperitoneal delivery of 25.0% mannitol resulted in undetectable GFP expression. There was no transduction of ependyma or choroid plexus with IA delivery, but neurons and astrocytes were transduced in a widely dispersed distribution in cortex, basal ganglia, thalamus, and brainstem.

rAAV-GFP and Mannitol Injection Into the Cerebral Ventricles Results in High Levels of Brain Expression

Prior reports demonstrated a benefit of systemic[24,25] or intraparenchymal[26] mannitol administration before direct brain delivery of AAV vectors. Because direct mannitol administration to the cerebral ventricles had never been tested, we compared systemic and ICV administration of 25.0% mannitol using a test dose of 10^{10} g.p. rAAV-GFP. Although both methods provided adequate levels of brain expression, co-injection of mannitol with vector directly into the ventricle in a 1:1 ratio was qualitatively superior in terms of vector spread and levels of gene expression (Figure 3). Injections without any mannitol whatsoever resulted mainly in choroid plexus expression with negligible brain expression, except along the

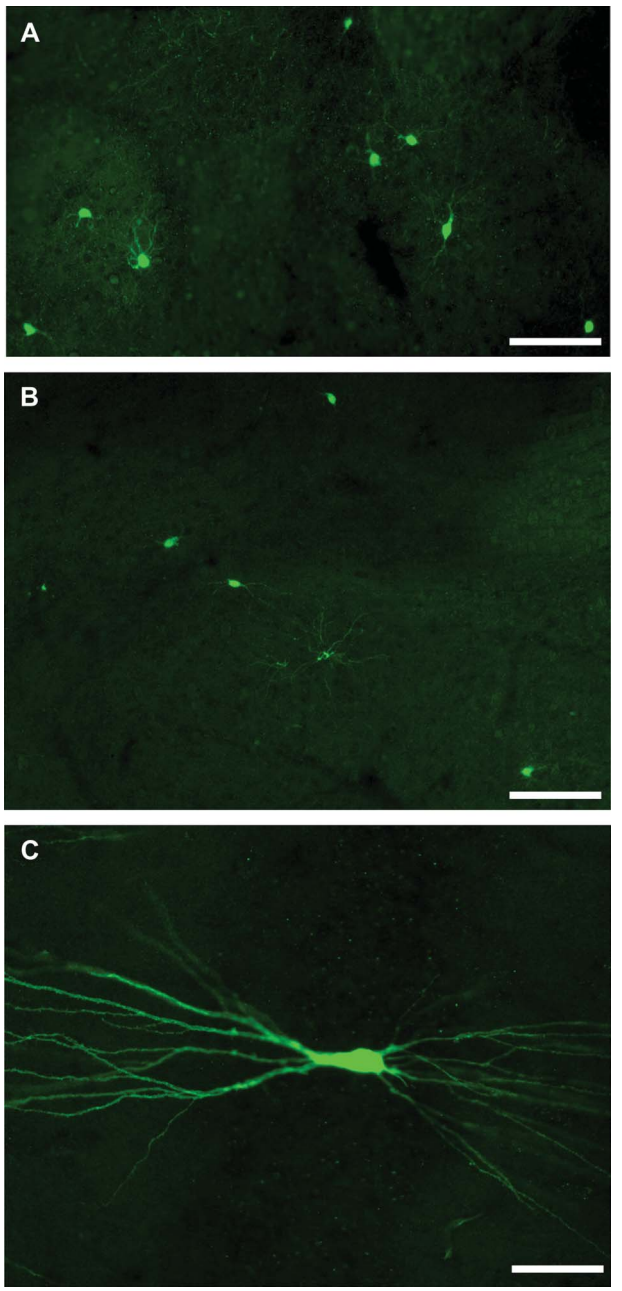

FIGURE 1. *Endovascular delivery of recombinant adeno-associated virus-green fluorescent protein (rAAV5-GFP) with 1.4-mol/L mannitol blood-brain barrier disruption. Initial experiments with low-dose (1×10^{10} genomic particles [g.p.]) vs high-dose (1×10^{12} g.p.) rAAV5-GFP and 1.4-mol/L mannitol gave virtually no transduction with low-dose treatment but scattered transduction with high-dose treatment, shown here. Widely scattered neurons are visible in (A) cortex, (B) basal ganglia, and (C) hippocampus after high-dose rAAV5-GFP and 1.4-mol/L mannitol blood-brain barrier disruption. Scale bars = 80 μm, 100 μm, and 50 μm, respectively.*

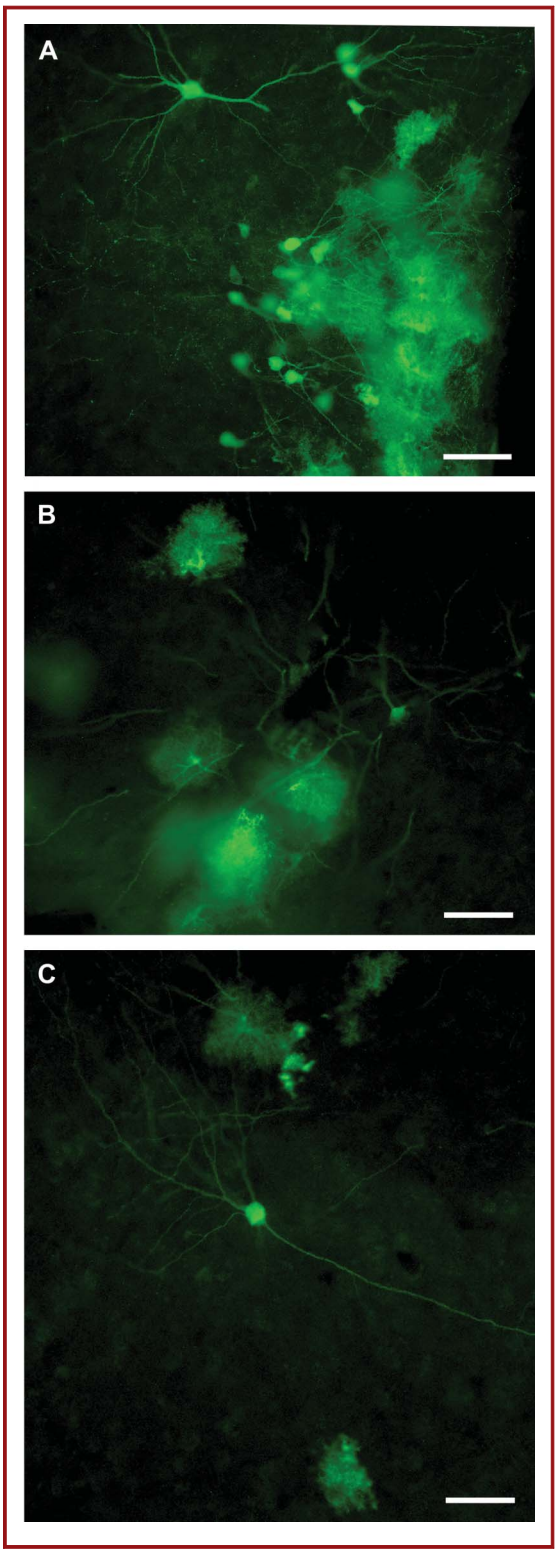

FIGURE 2. *Endovascular delivery of recombinant adeno-associated virus-green fluorescent protein (rAAV5-GFP) with 1.6-mol/L mannitol blood-brain barrier disruption. Subsequent experiments with high-dose (1 × 10^{12} g.p.) rAAV5-GFP and 1.6-mol/L mannitol blood-brain barrier disruption demonstrated superior transduction compared to 1.4-mol/L mannitol. Mucopolysaccharidosis type I mouse cortex 1 month after endovascular delivery to the right carotid artery (A-C). GFP-positive perivascular astrocytes are visible, along with surrounding multipolar neurons. With this delivery method, we targeted mainly cortical and basal ganglia neurons, plus an almost equal number of astrocytes and occasional microglia. Scale bars = 50 μm.*

needle tract. Although direct administration of mannitol to the ventricle was equivalent or better, in later experiments using ICV delivery with rAAV5-IDUA, for practical reasons (ie, to avoid the possibility of mannitol precipitation and blockage of the needle), we chose to use systemic mannitol pretreatment but with a 10-fold higher concentration of vector (10^{11} g.p.).

Qualitative rAAV5-IDUA Gene Expression In Vivo and Effects on Ganglioside Inclusions

After establishing dosing parameters with rAAV5-GFP, we proceeded to test the therapeutic vector rAAV5-IDUA, comparing IA and ICV delivery. Using monoclonal antibody for human IDUA, we observed high-level, widespread immunohistochemical expression of IDUA after ICV delivery of rAAV5-IDUA (Figure 4) over a 10-mm rostral-caudal distance. Cerebellum and spinal cord were not primary targets and were not examined. We compared the relative abundance of ganglioside GM3 inclusions in areas of high and low IDUA expression and found that neuronal inclusions decreased in direct proportion to the amount of IDUA produced. This was particularly evident with ICV delivery (Figure 4), but direct comparison of ICV and IA delivery showed that both methods were effective in lowering inclusions (Figures 5 and 6). However, GM3 brain pathology with IA delivery was improved only on the ipsilateral side, suggesting that bilateral carotid injections would be necessary for clinical application; these results were consistent with our quantitative assays for enzyme activity and GAG content that showed increased enzyme activity and decreased GAG only on the ipsilateral side. We also looked at the peripheral effects of rAAV5-IDUA treatment and as expected, IA delivery had the advantage of high expression in affected viscera compared with ICV delivery where the peripheral organs showed close to zero IDUA expression (Figure 7). This visceral expression is an additional potential benefit of IA treatment in MPS-I, which invariably shows hepatosplenomegaly.

Quantitative Effects of Gene Therapy on IDUA Enzyme Activity and GAG Levels

After immunohistochemical confirmation that human IDUA was present in treated IDUA-null homozygotes, together with

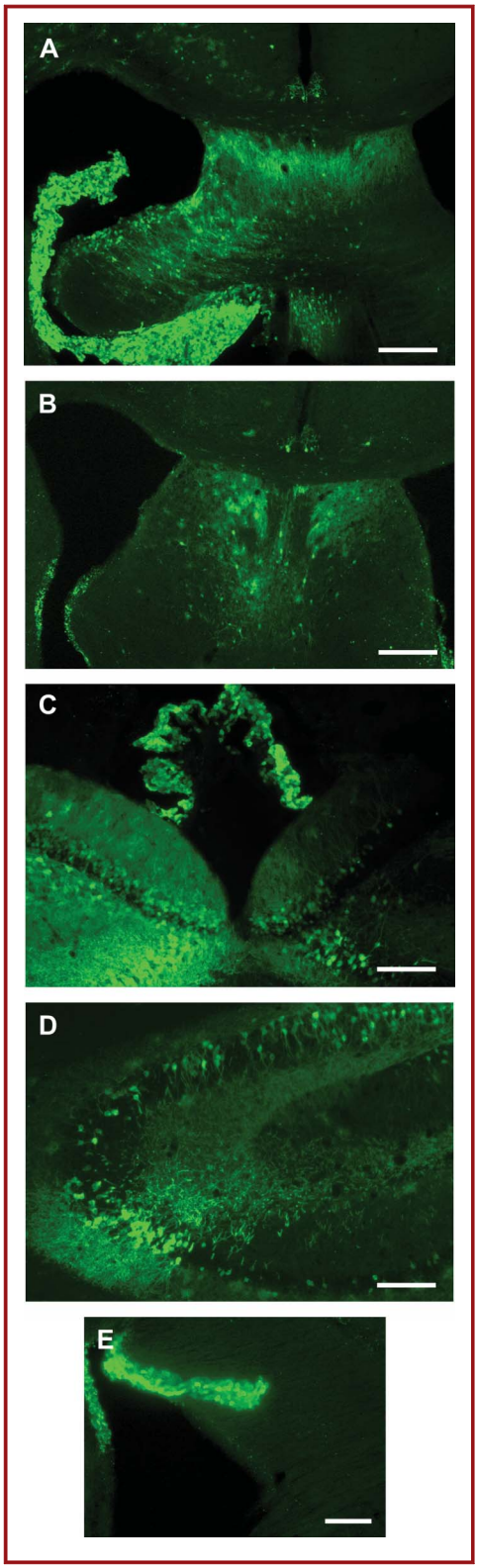

FIGURE 3. *Intracerebral ventricular (ICV) delivery of recombinant adeno-associated virus-green fluorescent protein (rAAV5-GFP) with mannitol co-administration, shown here at 1 month post-treatment.* **A**, *coronal view through corpus callosum and lateral ventricles in an animal treated with ICV delivery of rAAV5-GFP vector (10^{10} genomic particles [g.p.]) and 25% mannitol administered systemically. There is bilateral expression of GFP in neurons and astroglial, with intense choroid plexus staining. Scale bar = 300 μm.* **B**, *ICV delivery of rAAV5-GFP vector (10^{10} g.p.) with 25% mannitol co-administered along with vector directly to the cerebral ventricle in 1:1 ratio, showing ependymal and subependymal staining, also with strong neuronal staining and scattered astrocytes around white matter tracts. Scale bar = 300 μm.* **C**, *the same animal with ICV mannitol is shown through a section of the third ventricle, with strong GFP signal in the choroid plexus at the center and in dentate gyrus bilaterally. Compared with systemic mannitol, ICV mannitol showed slightly greater spread of vector and more intense neuronal staining. Scale bar = 180 μm.* **D**, *the same animal had extensive GFP transduction in CA2 and CA3, with robust bilateral hippocampal expression after a unilateral injection to the lateral ventricle. Scale bar = 70 μm.* **E**, *animals treated with ICV injection but without mannitol had expression in the choroid plexus, limited ependymal staining, and negligible intraparenchymal staining. Scale bar = 300 μm.*

qualitatively less GM3 inclusions, we performed quantitative analyses of tissue homogenates for enzyme activity, which showed significantly elevated IDUA activity in animals treated with ICV delivery, > 200% of the normal wild-type level. Enzyme activity was present in the ipsilateral hemisphere after IA delivery and at lower levels in the contralateral side (Figure 8). We found that GAG was significantly and bilaterally decreased in the animals treated with ICV injection and ipsilaterally decreased in the IA case (Figure 9). Together with the decrease in neuronal inclusions, these data suggest a physiologically relevant effect on brain pathology with both delivery techniques and show that a modest level of enzyme activity (≤ 42% of wild type) is sufficient to reverse the pathological process.

DISCUSSION

This study provides evidence that IA and ICV delivery approaches are both feasible for in vivo gene therapy of Hurler disease. Although gene expression with ICV delivery was superior to IA delivery, we found that different cell types and regions were targeted. In initial experiments using rAAV5-GFP vector with ICV delivery but without mannitol, the choroid plexus was uniformly well stained but ependymal cells were significantly less so, and there were only scattered neurons outside the periventricular area. Conversely, with ICV delivery with systemic mannitol or direct infusion of mannitol to the lateral ventricle in a 1:1 ratio, neurons and astrocytes were also transduced in a widespread distribution

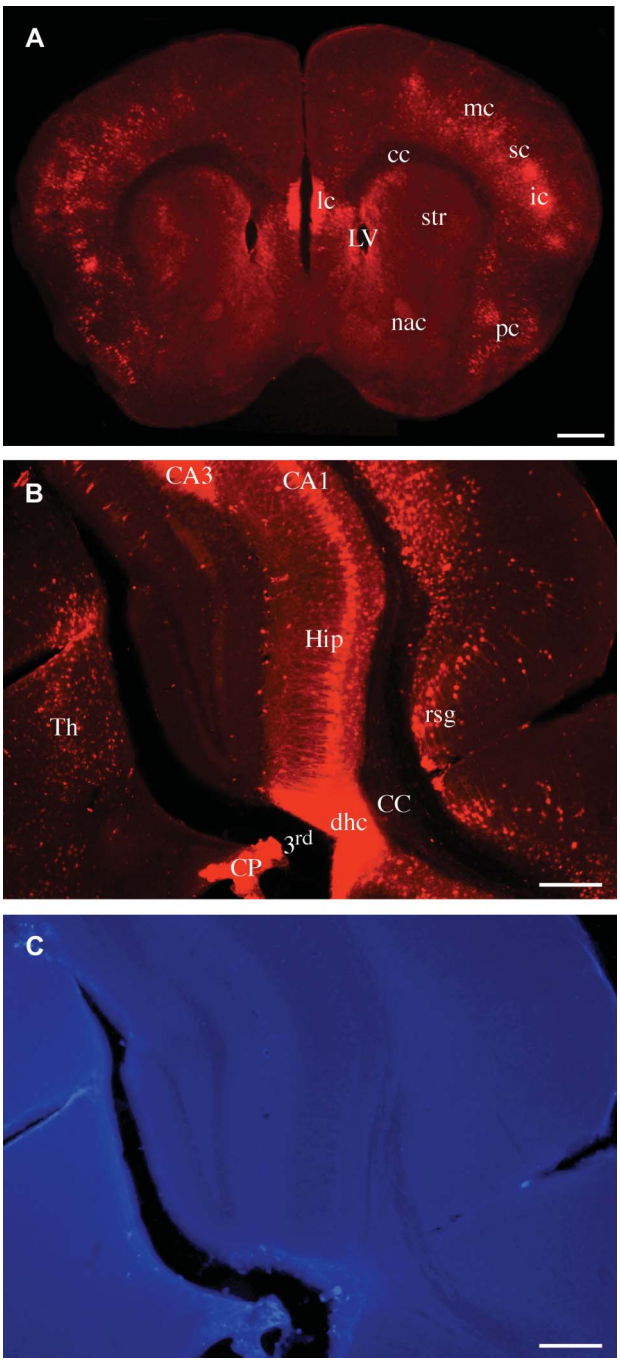

FIGURE 4. *Global expression of α-L-iduronidase (IDUA) in the mucopolysaccharidosis type I (MPS-I) brain with intracerebral ventricular (ICV) delivery. Coronal views of the MPS-I mouse brain treated with recombinant adeno-associated virus (rAAV5)-IDUA (10^{11} genomic particles) via the ICV route with systemic 25% mannitol co-administration, demonstrating high-level staining throughout the cerebrum and subcortical areas.* **A**, *representative section from the anterior cerebrum, with intense staining to human IDUA in diverse areas, including motor cortex (mc), sensory cortex (sc), granular insular cortex (ic), piriform cortex (pc), limbic cortex (lc), striatum (str), and nucleus accumbens (nac). Also shown are the lateral ventricle (LV) and corpus callosum (cc). Scale bar = 1 mm.* **B**, *representative section from posterior cerebrum, with intense staining to human IDUA in the choroid plexus (CP), dorsal hippocampal commissure (dhc), retrosplenial granular cortex (rsg), hippocampus (Hip) including CA1 and CA3, and thalamus (Th). Also shown are corpus callosum (CC) and third ventricle (3^{rd}). Scale bar = 250 μm.* **C**, *immunohistochemical double labeling was used to look for the ganglioside GM3 and showed virtually zero inclusions, consistent with very high levels of IDUA.*

throughout the cortex, basal ganglia, thalamus, and brainstem. Because our biodistribution studies with rAAV5-GFP and prior published work[24,29] have indicated that brain expression was very limited using rAAV5 with ICV delivery but without mannitol, follow-up experiments with rAAV-IDUA were performed using an optimized mannitol protocol. Although we cannot rule out the possibility that some of the biochemical correction after ICV delivery with rAAV5-IDUA came from transduced choroid plexus cells, the number of those cells compared with the number of neurons transduced by this method was quite small, and parenchymal transduction clearly dominates.

With the high-dose IA delivery paradigm using rAAV5-GFP or rAAV-IDUA and blood-brain barrier disruption with an optimized 29.1% mannitol protocol, we observed widespread cortical, hippocampal, and basal ganglia expression in neurons and an almost equal number of astrocytes and scattered microglia in the ipsilateral hemisphere but with much lower levels of overall gene expression. In terms of potential clinical translation, 29.1% mannitol is currently not approved by the Food and Drug Administration, although the original data of Rapoport et al[18] showed that higher concentrations (including supersaturated mannitol) are superior for disruption. It should be noted that clinical blood-brain barrier disruption uses hyperosmolar contrast dye for catheter angiography, which may also contribute to blood-brain barrier disruption effects, and lesser quantities of hyperosmotic solutions would likely be necessary in large animals or humans for disruption. In any case, we found that mannitol dosing at approximately 4 g/kg for blood-brain barrier disruption and 5 g/kg for systemic delivery in mice was not toxic and in humans would be predicted to be dose limited only by renal effects. Used in conjunction with ICV vector delivery, direct ICV delivery of mannitol at a 100-fold lower dose (1:1 by volume with rAAV vector) was found to have similar effects on vector spread and should have close to zero systemic effects.

The advent of self-complementary adeno-associated viral vectors (scAAV) has reinvigorated interest in endovascular gene therapy.[34,35]

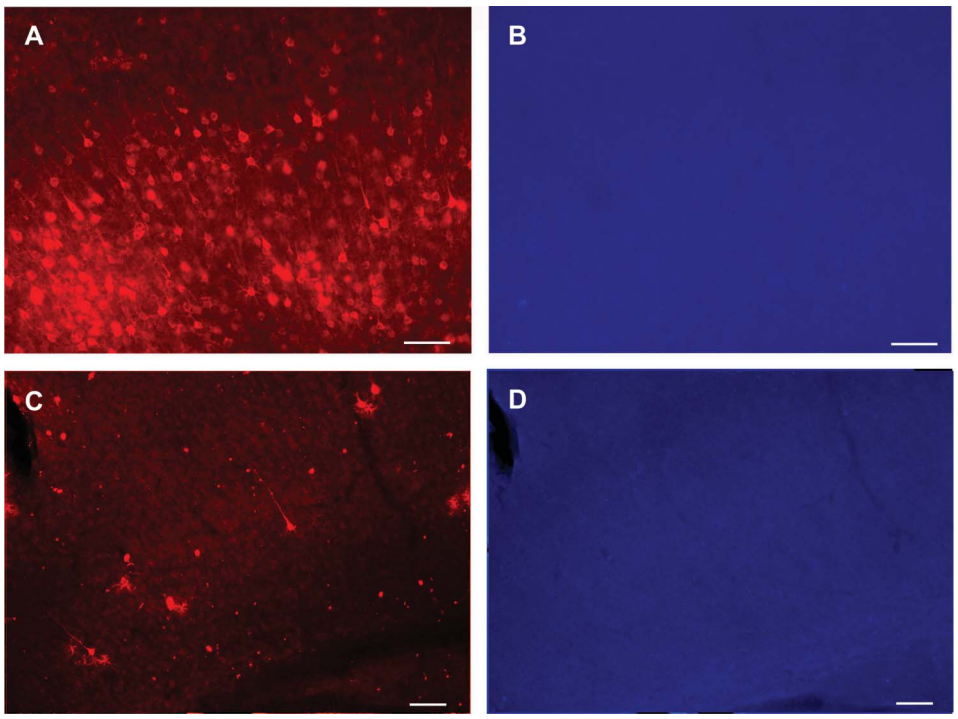

FIGURE 5. *Comparison of intraventricular and endovascular α-L-iduronidase (IDUA) expression in the frontal lobe. Representative areas of frontal lobe were compared after intracerebral ventricular (ICV) and intra-arterial (IA) delivery of therapeutic vector in homozygous mucopolysaccharidosis type I animals.* **A**, *recombinant adeno-associated virus (rAAV5)-IDUA at 10^{11} genomic particles (g.p.) via ICV delivery with systemic 1.4-mol/L mannitol resulted in high-level transduction primarily in neurons, with (***B***) concomitant lack of GM3 neuronal inclusions by double labeling.* **C**, *rAAV5-IDUA delivered at 10^{12} g.p. via IA delivery with 1.6-mol/L mannitol blood-brain barrier disruption showed mixed neuronal and astrocyte expression in the same region, with (***D***) equivalent decrease in GM3 in this region, despite a much lower level of cellular transduction. IDUA-positive cells were found throughout the frontal lobe with both delivery techniques, although the contralateral endovascular case had far fewer IDUA-positive cells than the ipsilateral endovascular case. Scale bars = 100 μm.*

The transduction efficiency of scAAV is at least 20 times greater than single-stranded vectors, and for reasons that are poorly understood, scAAV has been shown to express transgenes in the brain even in the absence of standard blood-brain barrier disruption. Despite superior transduction efficiency, a practical issue is the limited packaging size of scAAV, half that of single-strand DNA vectors (2.1-kb maximum transgene size as opposed to 4.2 kb), so some therapeutic genes cannot be packaged. Over a decade ago, it was shown that a bolus of intravenous mannitol (2 g/kg) together with scAAV2 vectors in mice resulted in low-level brain expression of a transgene.[36,37] Other serotypes such as AAV9, AAVrh39, AAVrh43,[38-40] and engineered vectors[41,42] showed even greater ability to cross the blood-brain barrier, even without hyperosmolar treatment. Mannitol appears to potentiate brain gene expression of rAAV vectors, whether single-strand or self-complementary,[43,44] and our data suggest that it is mandatory for widespread rAAV5 expression in the brain with IA or ICV delivery. The vector serotype, route of delivery, animal species, and developmental age also affect the ability of rAAV to cross the blood-brain barrier and the cell types transduced.

Prior studies with rAAV5 reported variable distributions of transduced cells, suggesting that gene expression depends in part on the method of administration. For example, rAAV5 with CBA promoter injected directly into the hippocampus transduced the entire hippocampus (including CA1-CA3 and dentate gyrus), and there was also retrograde transport to the medial septal nucleus,[45] but astrocytes were not effectively transduced. In another study, ICV delivery of rAAV5 (3×10^{10} g.p.) using a marker gene showed mainly ependymal expression and only a small amount of positive neurons and astrocytes ($< 5\%$) in the striatum or other areas, similar to what we observed without mannitol.[29] In our study, using ICV delivery with adjuvant mannitol, we observed high-level transduction in neurons, ependyma, and choroid plexus. When IA delivery was used with 29.1% mannitol blood-brain barrier disruption, expression was found in neurons and astrocytes (ratio was at least 2:1), and zero expression was found in ependyma or

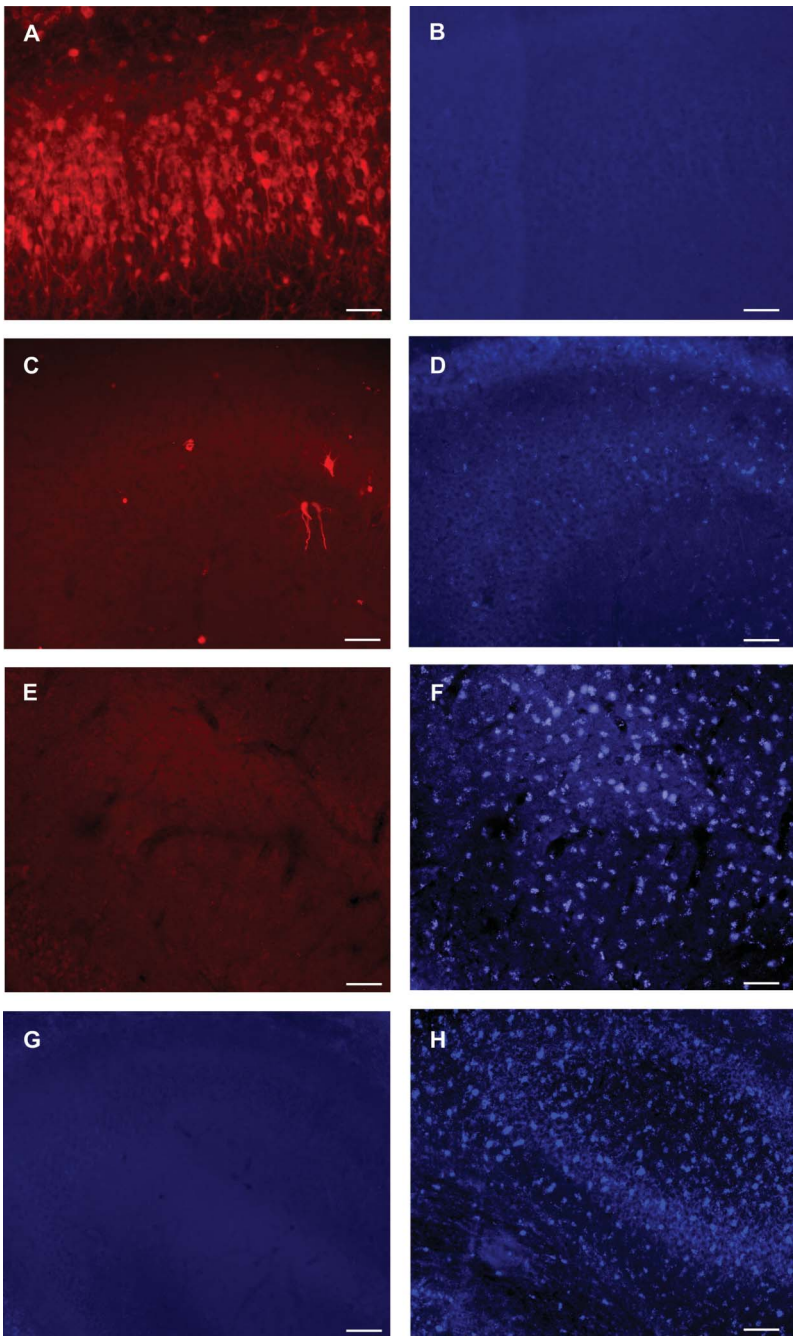

FIGURE 6. *Comparison of intraventricular and endovascular α-L-iduronidase (IDUA) expression in hippocampus. Representative sections through the hippocampus in mucopolysaccharidosis type I (MPS-I) animals treated with recombinant adeno-associated virus (rAAV5)-IDUA through the intracerebral ventricular (ICV) or IA route, showing relative transduction efficiency and monosialodihexosylganglioside (GM3) neuronal inclusions.* **A**, *ICV rAAV5-IDUA with systemic 1.4-mol/L mannitol shows* (**B**) *corresponding lack of GM3 inclusions by double labeling.* **C**, *endovascular delivery with 1.6-mol/L mannitol blood-brain barrier disruption shows IDUA-positive scattered neurons and* (**D**) *weak staining to GM3. The same endovascularly treated animal in the contralateral hemisphere* (**E**) *shows an absence of IDUA-positive cells and corresponding presence of GM3 neuronal inclusions* (**F**), *suggesting a lack of treatment effect on the contralateral side. GM3 for wild-type negative control* (**G**) *and age-matched MPS-I positive control* (**H**) *is shown for comparison. Scale bars = 80 μm.*

choroid plexus. Other studies in MPS-I mice have used rAAV2 or rAAV5 with intrathecal[46] (but not ICV) or intraparenchymal[47] delivery, but they did not quantify cellular distribution, and enzyme activities were variable. A study in MPS-I dogs using rAAV5 (4.8×10^{11} g.p.) with intraparenchymal injections reported phenotypic correction with relatively low enzyme activity in 6 of 7 animals treated. That canine study used only half the dose we applied to each mouse with IA delivery, which suggests that more aggressive dosing is possible in large animals or humans.[48]

There has been excitement about reports of global delivery to the brain after intravenous delivery of scAAV vectors in mice without hyperosmolar treatment. At the same time, there was debate as to why rAAV injections into the facial vein or superior temporal vein in neonatal animals gave different expression patterns than injections into the tail vein in adult animals.[33] Some proposed that this discrepancy is the result of an immaturity of the blood-brain barrier, because astrocyte formation occurs in the early postnatal period in mice and astrocyte endfeet are not fully developed until 3 weeks of age. As a result, rAAV vector, which achieves transcellular or paracellular transport in the neonatal period, would not be limited by the surrounding cells and could spread to neurons. Yet, vascular endothelial tight junctions are fully developed in neonates and the blood-brain barrier is intact, and it was proposed that certain gene therapy delivery paradigms may describe "a mechanical reaction when a physiological system is abused, rather than being a manifestation of a developmental phenomenon."[49] For example, studies that reported high-level neuronal transduction in the brain after high-volume intravenous delivery of scAAV to neonatal mice[38,39] did not include controls to examine the effects on the integrity of the blood-brain barrier.

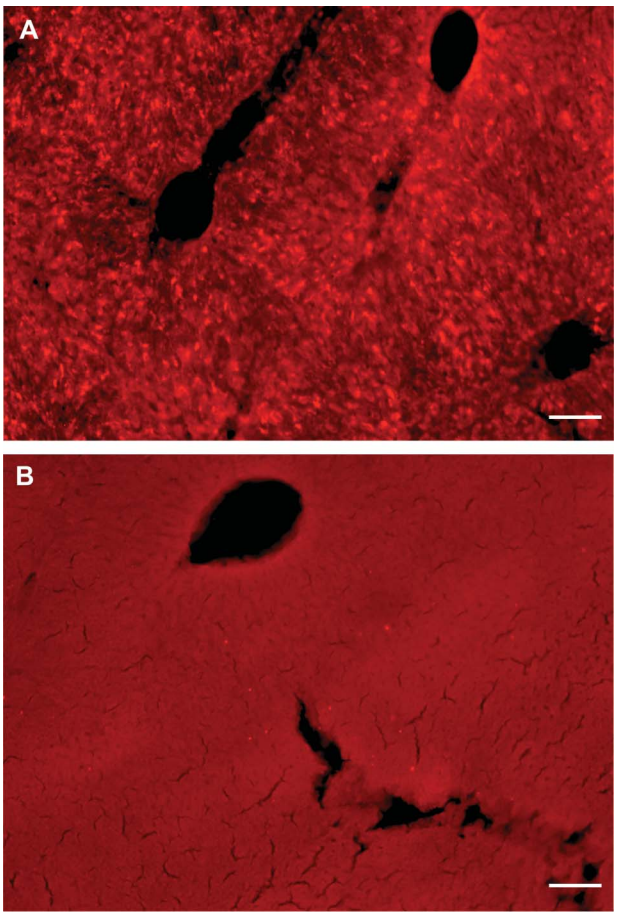

FIGURE 7. *Spread of recombinant adeno-associated virus–α-L-iduronidase (rAAV5- IDUA) to viscera.* **A**, *with endovascular delivery, the majority of vector did not penetrate the blood-brain barrier and was shunted to other organs such as the liver and spleen. There is strong positive staining to human IDUA around liver sinusoids. With intraventricular delivery (***B***), the vast majority of the vector stayed in the central nervous system. As a result, there was close to zero IDUA-positive staining in viscera. With either route, there was also minimal uptake of rAAV5 in spleen (data not shown), except for isolated blood-derived cells. Scale bars = 100 μm.*

In our study, although we used a fairly large injectate to the ventricle, equivalent to a 28-mL injection to the adult human, we drained off close to that volume before injection and replaced it with vector/mannitol mixture. On the other hand, neonatal mouse experiments have commonly injected enormous volumes that would certainly be injurious if proportional doses were administered to humans. This highlights the need for physiologically relevant animal models, not merely those that obtain the most impressive results, and data on rAAV expression and cellular tropism should be put into the context of the delivery paradigm and possible effects on the blood-brain barrier or other structures.

Large-animal studies have not yet clarified how to achieve global neuronal transduction with rAAV. In a study by Samaranch et al[50] at the University of California, San Francisco, 3 primates were injected via the carotid artery with scAAV9-GFP (3×10^{13} g.p.) without blood-brain barrier disruption or mannitol. They found a low level of scattered expression of GFP in 2 of 3 animals and none in 1 of 3, which was later found to have a neutralizing antibody titer to AAV9. This study showed that scAAV9 was able to enter the brain when very high titers were used, even without mannitol or mechanical disruption of the blood-brain barrier. However, expression levels were low in scattered astrocytes and neurons. Using an intracisternal paradigm, they achieved much better results, including transduction of many neurons. Gray et al[51] similarly delivered scAAV9 or scAAV2.5 (engineered virus) at 2×10^{12} g.p. to the cisterna magna and reported transduction of approximately 2% of total brain cells, including approximately 50% of cells in certain brain structures when the cells were counted in a semi-quantitative fashion.

In another study with adult mice and primates injected intravenously with scAAV9-GFP, Gray et al[44] found that primarily astrocytes were transduced in the brain. In other large-animal studies, Bevan and coworkers[52] injected scAAV9-GFP (1×10^{14} g.p.) into the saphenous vein in primates in a 10-cm^3 volume and found transgene expression in the lower spinal cord. They looked at the brain in P1 through P90 animals and found significant cortical staining, but the vast majority of transduced cells were astrocytes or microglia. With intrathecal injections of scAAV9-GFP (5.2×10^{12} g.p.) in P5 baby

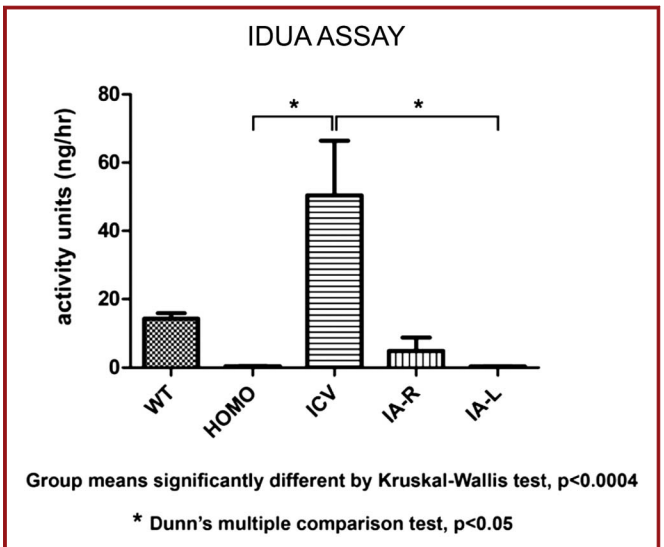

FIGURE 8. *Effects of gene therapy on quantitative α-L-iduronidase enzyme (IDUA) activity. Although expression of human IDUA was evident in immunohistochemistry, a quantitative activity assay was used to confirm that IDUA was biologically active in vivo. The ipsilateral endovascular treatment had a mean enzyme activity 42% of wild type (WT), and intracerebral ventricular (ICV) treatment had mean enzyme activity >200% of WT levels. Homozygous (null) animals had essentially zero enzyme activity. These results correlated with a relative decrease in monosialodihexosylganglioside and glycosaminoglycans in the same regions. For specific activity in nanograms per hour per 1 mg protein, divide the relative activity by 0.0675. Values shown are mean (SEM). HOMO, homozygous (IDUA null); IA-L, contralateral endovascular; IA-R, ipsilateral endovascular.*

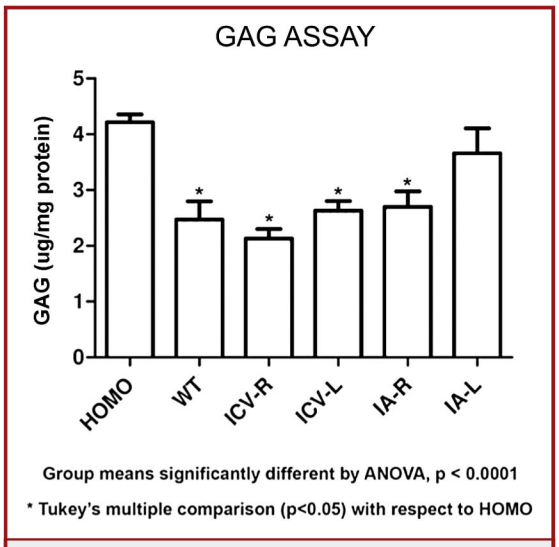

FIGURE 9. *Effects of gene therapy on quantitative glycosaminoglycan (GAG) content. Because the hallmark of mucopolysaccharidosis type I (MPS-I) is an increase in tissue GAG, we performed a quantitative assay to determine whether there was a decrease in GAG content after gene therapy. MPS-I animals that were treated with recombinant adeno-associated virus–α-L-iduronidase (IDUA) via the intraventricular route had GAG levels indistinguishable from those of wild-type animals, and the same was true for ipsilateral specimens from endovascularly treated animals. All conditions tested, except for the contralateral endovascular case, were significantly different from the MPS-I homozygotes ($P < .05$). Samples were normalized to total protein content. Values shown are mean (SEM). HOMO, homozygous (IDUA null); ICV-L, intracerebral ventricular contralateral; ICV-R, intracerebral ventricular ipsilateral; IA-L, contralateral endovascular; IA-R, ipsilateral endovascular; WT, wild type.*

pigs, they found transgene expression in "scattered cells near the meningeal surfaces" and a small amount in cerebellar Purkinje cells and some brainstem nuclei. The difference between excellent neuronal expression in mice and relatively poor neuronal expression in monkeys was attributed by the authors to differences in gliagenesis, but the most striking difference was actually the injection paradigm because young mice with facial vein injections had high-level neuronal transduction whereas older mice with tail vein injections and primates with peripheral intravenous injections demonstrated relatively low-level glial transduction. On the basis of these studies, intravenous scAAV9 transduces mainly astrocytes in the absence of a blood-brain barrier disruption, but delivery to the cerebrospinal fluid compartment results in variable amounts of neuronal transduction.

There are theoretical reasons to target ependyma and choroid plexus in MPS-I with ICV delivery, but IA treatment is well suited to treating the unique periadventitial pathology seen in MPS-I. Given the partial correction in GAG and GM3 that we observed, despite a relatively low level of IDUA-positive cells, there is a rationale to examine further the endovascular approach. Although ipsilateral mean IDUA enzyme in endovascularly treated animals was only 42% of wild type, the very high expression levels observed with intraventricular delivery (> 200% wild-type) may not be necessary for symptomatic improvement. In fact, only a minority of transduced cells (assuming a broad distribution in the brain) may be sufficient for phenotypic correction because of cross-correction by uptake of enzyme by neighboring cells through mannose-6-phosphate receptors,[53] perhaps as little as 5% to 10%. Hurler-Scheie patients (MPS-IS) have a mild phenotype as a result of mild mutations, with IDUA expression < 1% of normal.[54] Although our study was not designed to address phenotypic correction, other studies have verified that it occurs after rAAV gene therapy in the same mouse model.[55] In addition to biochemical and immunohistochemical correction, a major outcome of the study was that blood-brain barrier disruption and aggressive dosing with 1 trillion viral particles did not cause adverse effects on the animal's behavior, confirming the safety profile of rAAV5.

CONCLUSION

Of 1105 clinical gene therapy protocols submitted for US regulatory approval since the inception of human gene therapy in 1990, only a handful of in vivo studies have involved neurological

disorders, and none have targeted the mucopolysaccharidoses.[56] The main reasons for this underrepresentation of neurological disorders are the technical challenges of gene vector delivery to the brain and lack of consensus on the effectiveness of different delivery paradigms. Protocols for human CNS gene therapy with rAAV vectors to date have used direct intracerebral injections as a result of the perception of greater cellular transduction, despite the fact that less invasive methods are available. Recent long-term results in gene therapy for Canavan disease at up to 10 years post-treatment showed that multisite intracerebral delivery in humans with 1 g/kg intravenous mannitol can lead to clinical stabilization,[28] but neurosurgical delivery was associated with complications of hemorrhage and infection. Alternative delivery via IA or ICV and intracisternal methods may be safer and more efficacious. In terms of clinical translation, mannitol has a long clinical history of safety and efficacy in various neurosurgical applications, and we found that it optimizes the cerebral biodistribution of rAAV5 regardless of the delivery route. Differences in technical protocols—choice of vector, route of delivery, volume and rate of injection, and treatment with hyperosmolar agents—affect the efficiency of gene therapy, and more biodistribution studies are needed to make conclusions on which methods are best suited for human use.

Disclosures

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REFERENCES

1. Matalon R, Dorfman A. Hurler's syndrome, an alpha-L-iduronidase deficiency. *Biochem Biophys Res Comm.* 1972;47(4):959-964.
2. Bach G, Friedman R, Weissman B, Neufeld EF. The defect in Hurler and Scheie syndromes: deficiency of alpha-L-iduronidase. *Proc Natl Acad Sci U S A.* 1972;69(8):2048-2051.
3. Clarke JTR. Hurler's disease. In: *NORD Guide to Rare Disorders*. Philadelphia, PA: Lippincott; 2003.
4. Staba SL, Escolar ML, Poe M, et al. Cord blood transplants from unrelated donors in patients with Hurler's syndrome. *N Engl J Med.* 2004;350(19):1960-1969.
5. Unger ER, Sung JH, Manivel JC, et al. Male donor-derived cells in the brains of female sex-mismatched bone marrow transplant recipients: Y-chromosome specific in situ hybridization study. *J Neuropath Exper Neurol.* 1993;52(5):460-470.
6. Dekaban AS, Constantopoulos G. Mucopolysaccharidosis types I, IV, IIIA, V: pathological and biochemical abnormalities in the neural and mesenchymal elements of the brain. *Acta Neuropath.* 1977;39(1):1-7.
7. MacBrinn M, Okada S, Woollacott M, et al. Beta-galactosidase deficiency in the Hurler syndrome. *N Engl J Med.* 1969;281(7):338-343.
8. McGlynn R, Dobrenis K, Walkley SU. Differential subcellular localization of cholesterol, gangliosides, and glycosaminoglycans in murine models of mucopolysaccharide storage disorders. *J Comp Neurol.* 2004;480(4):415-426.
9. Love S, Louis D, Ellison D, eds. *Greenfield's Neuropathology*, 8th ed. London, UK: Hodder Arnold; 2008:544-547.
10. Traas AM, Wang P, Ma X, et al. Correction of clinical manifestations of canine mucopolysaccharidosis I with neonatal retroviral vector gene therapy. *Mol Ther.* 2007;15(8):1423-1431.
11. Janson CG, McPhee SW, Leone P, et al. Viral-based gene transfer to the mammalian CNS for functional genomic studies. *Trends Neurosci.* 2001;24(12):706-712.
12. Ohmi K, Greenberg DS, Rajavel KS, Ryazanysev S, Li HH, Neufeld EF. Activated microglia in cortex of mouse models of mucopolysaccharidoses I and IIIB. *Proc Natl Acad Sci U S A.* 2003;100(4):1902-1907.
13. Haskins ME, Aguirre GD, Jezyk PF, Desnick RJ, Patterson DF. The pathology of the feline model of mucopolysaccharidosis I. *Am J Path.* 1983;112(1):27-36.
14. Spellacy E, Shull RM, Constantopoulos G, Neufled EF. A canine model of human alpha-L-iduronidase deficiency. *Proc Natl Acad Sci U S A.* 1983;80(19):6091-6095.
15. Di Pasquale G, Davidson BL, Stein CS, et al. Identification of PDGFR as a receptor for AAV-5 transduction. *Nat Med.* 2003;9(10):1306-1312.
16. Boutin S, Monteilhet V, Veron P, et al. Prevalence of serum IgG and neutralizing factors against adeno-associated virus (AAV) types 1,2,5,6,8, and 9 in the healthy population: implications for gene therapy using AAV vectors. *Hum Gene Ther.* 2010;21(6):704-712.
17. Rapoport SI, Hori M, Klatzo I. Reversible osmotic opening of the blood-brain barrier. *Science*. 1971;173(4001):1026-1028.
18. Rapoport SI, Fredericks WR, Ohno K, Pettigrew KD. Quantitative aspects of reversible osmotic opening of the blood-brain barrier. *Am J Physiol.* 1980;238(5):R421-R431.
19. Neuwelt EA, Frenkel EP, Diehl J, Vu LH, Rapoport S, Hill S. Reversible osmotic blood-brain barrier disruption in humans: implications for the chemotherapy of malignant brain tumors. *Neurosurgery*. 1980;7(1):44-52.
20. Doran SE, Ren XD, Betz AL, et al. Gene expression from recombinant viral vectors in the central nervous system after blood-brain barrier disruption. *Neurosurgery*. 1995;36(5):965-970.
21. Nilaver G, Muldoon LL, Kroll RA, et al. Delivery of herpesvirus and adenovirus to nude rat intracerebral tumors after osmotic blood-brain barrier disruption. *Proc Natl Acad Sci U S A.* 1995;92(21):9829-9833.
22. Muldoon LL, Nilaver G, Kroll RA, et al. Comparison of intracerebral inoculation and osmotic blood-brain barrier disruption for delivery of adenovirus, herpesvirus, and iron oxide particles to the brain. *Am J Path.* 1995;147(6):1840-1851.
23. Liu R, Martuza RL, Rabkin SD. Intracarotid delivery of oncolytic HSV vector G47Delta to metastatic breast cancer in the brain. *Gene Ther.* 2005;12(8):647-654.
24. Leone P, Janson CG, Bilaniuk L, et al. Aspartoacylase gene transfer to the mammalian central nervous system with therapeutic implications for Canavan disease. *Ann Neurol.* 2000;48(1):27-38.
25. Ghodsi A, Stein C, Derksen T, Martins I, Anderson RD, Davidson BL. Systemic hyperosmolality improves beta-glucuronidase distribution and pathology in murine MPS VII brain following intraventricular gene transfer. *Exp Neurol.* 1999;160(1):109-116.
26. Burger C, Nguyen FN, Deng J, Mandel RJ. Systemic mannitol-induced hyperosmolality amplifies rAAV2-mediated striatal transduction to a greater extent than local co-infusion. *Mol Ther.* 2005;11(2):327-331.
27. Janson C, McPhee S, Bilaniuk L, et al. Clinical protocol: gene therapy of Canavan disease: AAV2 vector for neurosurgical delivery of aspartoacylase gene (ASPA) to the human brain. *Hum Gene Ther.* 2002;13(11):1391-1412.
28. Leone P, Shera D, McPhee SW, et al. Long-term follow-up of gene therapy for Canavan disease. *Sci Transl Med.* 2012;4(165):165ra163.
29. Davidson BL, Stein CS, Heth JA, et al. Recombinant adeno-associated virus type 2, 4, and 5 vectors: transduction of variant cell types and regions in the mammalian central nervous system. *Proc Natl Acad Sci U S A.* 2000;97(7):3428-3432.
30. Scott HS, Anson DS, Orsborn A M, et al. Human alpha-L-iduronidase: cDNA isolation and expression. *Proc Natl Acad Sci U S A.* 1991;88(21):9695-9699.
31. Hartung SD, Frandsen JL, Pan D, et al. Correction of metabolic, craniofacial, and neurologic abnormalities in MPS I mice treated at birth with adeno-associated virus vector transducing the human alpha-L-iduronidase gene. *Mol Ther.* 2004;9(6):866-875.
32. Garcia-Rivera MF, Colvin-Wanshura LE, Nelson MS, et al. Characterization of an immunodeficient mouse model of mucopolysaccharidosis type I suitable for preclinical testing of human stem cell and gene therapy. *Brain Res Bull.* 2007;74(6):429-438.
33. Hopwood JJ, Muller V, Smithson A, Baggett N. A fluorometric assay using 4-methyl-umbelliferyl alpha-L-iduronide for the estimation of alpha-L-iduronidase activity and the detection of Hurler and Scheie syndromes. *Clinca Chim Acta.* 1979;92:257-265.
34. McCarty DM, Monahan PE, Samulski RJ. Self-complementary recombinant adeno-associated virus (scAAV) vectors promote efficient transduction independently of DNA synthesis. *Gene Ther.* 2001;8(16):1248-1254.

35. McCarty DM, Fu H, Monahan PE, Toulson CE, Naik P, Samulski RJ. Adeno-associated virus terminal repeat (TR) mutant generates self-complementary vectors to overcome the rate-limiting step to transduction in vivo. *Gene Therapy.* 2003;10 (26):2112-2118.
36. Fu H, Muenzer J, Samulski RJ, et al. Self-complementary adeno-associated virus serotype 2 vector: global distribution and broad dispersion of AAV-mediated transgene expression in mouse brain. *Mol Ther.* 2003;8(6):911-917.
37. McCarty DM, DiRosario J, Gulaid K, Muenzer J, Fu H. Mannitol-facilitated CNS entry of rAAV2 vector significantly delayed the neurological disease progression in MPS-IIIB mice. *Gene Ther.* 2009;16(11):1340-1352.
38. Zhang H, Yang B, Mu X, et al. Several rAAV vectors efficiently cross the blood-brain barrier and transduce neurons and astrocytes in the neonatal mouse central nervous system. *Mol Ther.* 2011;19(8)1440-1448.
39. Foust KD, Nurre E, Montgomery CL, Hernandez A, Chan CM, Kaspar BK. Intravascular AAV9 preferentially targets neonatal neurons and adult astrocytes. *Nat Biotechnol.* 2009;27(1):59-65.
40. 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.* 2011;28(3):271-276.
41. Chen YH, Chang M, Davidson BL. Molecular signatures of disease brain endothelia provide new sites for CNS-directed enzyme therapy. *Nat Med.* 2009;15 (10):1215-1219.
42. Dalkara D, Byrne LC, Lee T, et al. Enhanced gene delivery to the neonatal retina through systemic administration of tyrosine-mutated AAV9. *Gene Ther.* 2012;19 (2):176-181.
43. Fu H, Dirosario J, Killedar S, Zaraspe K, McCarty DM. Correction of neurological disease of MPSIIIB in adult mice by rAAV9 trans-blood-brain barrier gene delivery. *Mol Ther.* 2011;19(6):1025-1033.
44. Gray SJ, Matagne V, Bachaboina L, Yadav S, Ojeda SR, Samulski SJ. Preclinical differences of intravascular AAV9 delivery to neurons and glia: a comparative study of adult mice and nonhuman primates. *Mol Ther.* 2011;19(6):1058-1069.
45. Burger C, Gorbatyuk OS, Velardo MJ, et al. Recombinant AAV viral Vectors pseudotyped with viral capsids from serotypes 1, 2, and 5 display differential efficiency and cell tropism after delivery to different regions of the central nervous system. *Mol Ther.* 2004;10(12):302-317.
46. Watson G, Bastacky J, Belichenko P, et al. Intrathecal administration of AAV vectors for the treatment of lysosomal storage in the brains of MPS I mice. *Gene Ther.* 2006;13(11):917-925.
47. Desmaris N, Verot L, Puech JP, Caillaud C, Vanier MT, Heard JM. Prevention of neuropathology in the mouse model of Hurler syndrome. *Ann Neurol.* 2004;56(1):68-76.
48. Ciron C, Desmaris N, Colle MA, et al. Gene therapy of the brain in the dog model of Hurler's syndrome. *Ann Neurol.* 2006;60(2):204-213.
49. Saunders NR, Joakim EK C, Dziegielewska KM. The neonatal blood-brain barrier is functionally effective, and immaturity does not explain differential targeting of AAV9. *Nat Biotechnol.* 2009;27(9):804.
50. Samaranch L, Salegio EA, San Sebastian W, et al. Adeno-associated virus serotype 9 transduction in the central nervous system of nonhuman primates. *Hum Gene Ther.* 2012 23(4):382-389.
51. Gray SJ, Nagabhushan Kalburgi S, McGown TJ, Jude Samulski R. Global CNS gene delivery and evasion of anti-AAV neutralizing antibodies by intrathecal AAV administration in non-human primates. *Gene Ther.* 2013;20(4):450-459.
52. Bevan AK, Duque S, Foust KD, et al. Systemic gene delivery in large species for targeting spinal cord, brain, and peripheral tissues for pediatric disorders. *Mol Ther.* 2011;19(11):1971-1980.
53. Hartung SD, Reddy RG, Whitley CB, McIvor R S. Enzymatic correction and cross-correction of mucopolysaccharidosis type I fibroblasts by adeno-associated virus-mediated transduction of the alpha-L-iduronidase gene. *Hum Gene Ther.* 1999;10(13):2163-2172.
54. Scott HS, Litjens T, Nelson PV, et al. Identification of mutations in the alpha-L-iduronidase (IDUA) gene that cause Hurler and Scheie syndromes. *Am J Hum Genet.* 1993;53(5):973-986.
55. Wolf DA, Lenander AW, Nan Z, et al. Direct gene transfer to the CNS prevents emergence of neurologic disease in a murine model of mucopolysaccharidosis type I. *Neurobiol Dis.* 2011;43(1):123-133.
56. US Department of Health & Human Services, Office of Biotechnology Activities (OBA). *Documents Interest: RAC Protoc List.* 2012.