Towards Clinical Progress in the Mucopolysaccharidoses

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Overview
The National Institute of Neurological Disorders and Stroke (NINDS), the Office of Rare Diseases (ORD), and the National Institute of Diabetes and Digestive and Kidney Diseases at NIH along with the National MPS Society co-sponsored this workshop. This scientific workshop was organized by Mark Haskins, V.M.D., Ph.D. (University of Pennsylvania); Sissi Langford (National MPS Society); Catherine McKeon, Ph.D. (National Institute of Diabetes and Digestive and Kidney Diseases); Mark Sands, Ph.D. (Washington University School of Medicine); and Danilo A. Tagle, Ph.D. (National Institute of Neurological Disorders and Stroke)

The goals of the workshop include:

- The goal of this workshop is to identify and address impediments to effective therapies in the mucopolysaccharidoses (MPS)
- To develop a strategy to determine the minimum standard protocol for clinical application of effective therapies for MPS and other lysosomal storage disorders (LSDs).

Executive Summary
There was considerable progress reported at this workshop on both the clinical and basic research areas in the mucopolysaccharidoses (see agenda). The Panel Discussions also identified areas of research that are required for efficient translation of new therapies and approaches into the clinic.

Progress in basic and translational research:

A) Enzyme Replacement:
William Sly presented preliminary data showing that completely de-glycoslyated -glucuronidase had a dramatically increased half-life in serum and virtually eliminated lysosomal storage in the CNS of adult MPS VII mice. This surprising finding has obvious and important implications for the treatment of the CNS disease associated with MPS disorders.

Elizabeth Neufeld presented the principles behind the development of aptamer-based modifications of recombinant α-L-iduronidase for treatment of the CNS disease associated with MPS I. Preliminary data generated in an *in vitro* blood brain barrier model shows that this approach may be able to deliver enzyme across the blood-brain barrier (BBB).

Patricia Dickson showed promising data using intrathecal delivery of α-L-iduronidase in the canine model of MPS I. The levels of glycosaminoglycans in the brain were normalized and the enzyme persisted for 4-7 days.

B) Small Molecule Therapy:
Thomas Seyfried showed that treatment with NB-DGJ, which interferes with the synthesis of gangliosides, decreased the levels of gangliosides in the mouse models of GM1 gangliosidosis and Sandhoff disease. In addition, he showed that caloric restriction decreased inflammation, improved rotarod performance, and increased the life span of these mice, however, there was no reduction in gangliosides. Experiments are ongoing to determine the efficacy of combining substrate reduction and dietary restriction.

Steven Walkley showed that substrate reduction and by-product replacement therapies were partially effective in Niemann-Pick type C disease. He also showed that combining the two approaches was more effective than either therapy alone.

Frances Platt studied the effects of small molecule substrate reduction therapy combined with bone marrow transplantation, neuronal stem cells, or non-steroidal anti-inflammatory drugs. Each therapeutic approach synergized with substrate reduction therapy to varying degrees.

David Bedwell developed a murine model that contained an authentic human non-sense mutation leading to MPS I. Using this model he was able to test a safer (less toxic) analog of gentamycin for stop codon read-through. This safer drug was able to restore approximately 3% of the enzyme activity in vitro and reduced urinary glycoaminoglycans (GAGs) in vivo.

C) Stem Cells
Kyuson Yun showed that fetal liver cells cultured with Noggin resulted in a 5- fold increase in the generation of neuronal stem cells. Small numbers of these cells were seen in the brain following intracranial injection of transduced cells. Data was also presented describing an easily accessible source (nasal epithelium) of autologous neuronal stem cells that express the correct neuronal markers *in vitro*.

Raquel Walton presented in vitro data suggesting that there is a block in the maturation of neuronal stem cells derived from several different areas of the MPS VII dog brain. When transplanted into the brains of MPS VII dogs, only GUSB-positive astrocytes were observed and no GUSB-positive neurons were detected.

Nobuko Uchida presented pre-clinical data showing that human neuronal stem cells distributed widely throughout the neuraxis and reduced the clinical signs associated with the Shiverer mouse. The cells also distributed similarly throughout the brains in the mouse model of Infantile Batten Disease.

D) Gene Therapy:
Miguel Sena-Esteves showed that direct intracranial injection of either AAV1 or AAV8 reduced ganglioside and cholesterol accumulation in the brains of GM1 gangliosidosis mice. Retrograde transport of -galactosidase was observed with both vectors, and enzyme activity was observed as distant as the retina and spinal cord following AAV8 injection.

Gordon Watson showed that intrathecal injection of an AAV vector resulted in persistent expression and widespread reduction of lysosomal storage throughout the CNS in both the MPS VII and MPS I mouse models.

John Wolfe showed that intracranial injection of an AAV1 vector into the brain of -mannosidosis cats resulted in widespread correction of disease, and dramatic clinical improvement of the animals. He also showed that a 1 l injection of AAV in the ventral tegmental area, which sends projections to many areas of the brain, can deliver enzyme and reduce lysosomal storage throughout the brain in MPS VII mice.

Kathy Ponder extended her studies using retroviral-mediated gene therapy in newborn MPS I dogs and showed that in addition to correction in visceral organs, other tissues that have been refractory to conventional therapies (aorta and brain) also respond to high expression during the newborn period. Alberto Auricchio, using MPS VI rats and cats, showed that AAV1 and AAV8 expressing arylsulfatase B transduced the muscle and liver, respectively. He showed that each target tissue expressed high levels of enzyme but arylsulfatase B was not secreted from muscle. Data describing the clinical response is still pending.

Jay Dritz showed pre-clinical data comparing the levels of expression of GUSB and GUSB-TAT in the hematopoietic compartment following lentiviral mediated gene therapy. High level expression in multiple tissues was observed. Effects on critical tissues such as the brain and kidney are pending. Jean-Michel Heard showed that there was persistent and widespread expression of α-L-iduronidase in the brains of MPS I dogs following 8 separate injections of an AAV5 vector. Lysosomal storage was reduced in targeted areas of the brain. However, immunosuppression (cyclosporine) was required to maintain expression.

Matthew Ellinwood presented similar data in the canine model of MPS IIIB. Three affected but pre-symptomatic dogs were injected intracranially with an AAV5 vector and were maintained on an immunosuppressive regimen. There was widespread enzyme distribution and their neurological exam was normal at sacrifice, however, significant inflammatory lesions were seen in the absence of immunosuppression.

Xiucui Ma presented data showing that long term expression of α-L-iduronidase was possible in dogs with MPS I treated as young adults following transient treatment with anti-CD4 or anti-CD40 ligand combined with CTLA-4. Interestingly, data was also presented that showed improvements in skeletal disease, auditory-evoked brain stem responses, and decreased storage in the brain. Adarsh Reddy showed that bone marrow transplantation or CNS-directed AAV-mediated gene therapy alone in the murine model of globoid-cell leukodystrophy resulted in significant increases in life span to an average of 45 and 55 days of age, respectively. However, when combined the two disparate therapies synergized dramatically and resulted in an average life span of 105 days.

Mark Sands presented follow- up data on the original observation of hepatocellular carcinoma following systemic AAV-mediated gene therapy. They repeated the original observation in a larger cohort of animals and showed that all of the AAV proviral sequences integrated in a region of mouse chromosome 12 that contains a large number of microRNAs. These integration events disrupted the normal expression pattern in that region.

E) Animal Models:
Andrea Ballabio discussed the characterization of the multiple sulfatase deficiency mice. This mouse has a very severe phenotype which includes systemic inflammation, neurodegeneration, and a defect in autophagy.

Progress in clinical research:

A) Enzyme Replacement:
Three recombinant protein products are currently approved for clinical use for enzyme replacement therapy in MPS I (Aldurazyme), MPS II (Elaprase) and MPS VI (Naglazyme). In general, the clinical response following intravenous infusion has been positive with reduced hepatosplenomegally, increased range of motion, increased stamina, and decreased urinary GAGs. Enzyme replacement products are in development for MPS IV and MPS VII.

Patricia Dickson discussed the preliminary findings of a human clinical trial to test the safety of intrathecal delivery of α-L-iduronidase. Three patients have been entered into the trial. So far the drug seems to be well tolerated with no severe adverse reactions and two patients have shown some symptomatic improvement.

B) Gene Therapy:
Ron Crystal discussed the preliminary data from an AAV2-mediated CNS-directed gene therapy clinical trial in children with Late Infantile Neuronal Ceroid Lipofuscinosis. So far, the procedure has been well tolerated and there is preliminary evidence that the procedure may be providing some efficacy. He also outlined improvements to the current protocol, specifically, the use of a new AAV serotype (rh from rhesus) that results in higher level expression and distribution in the brain. Jean-Michel Heard outlined an AAV5-mediated CNS-directed human gene therapy clinical trial for MPS I. This trial is based on the pre-clinical data generated in the canine model of MPS I.

C) Stem Cell-Mediated Therapy:
Nobuko Uchida presented preliminary data regarding a human clinical trial for Infantile Neuronal Ceroid Lipofuscinosis using neuronal stem cells. Several children have been injected and there have been no severe adverse reactions as of yet. Clinical evaluation is pending.

Joanne Kurtzberg discussed the use of umbilical cord blood for the treatment of lysosomal disorders. They have clear evidence that early treatment (as early as several weeks or months of age) is critical for effective therapy. They have also initiated a trial where they have added accessory cells that enhance early engraftment (data are pending).

D) Newborn Screening and developing Outcome Measures
Ronald Scott and John Hopwood gave updates on the progress with comprehensive newborn screening for lysosomal storage diseases. John Hopwood discussed his plans for screening 100,000 newborns in Australia with his mass spectrometry approach.

Joe Muenzer, Chet Whitley and Maria Escolar discussed the importance of meaningful clinical outcomes and natural history collection for the evaluation of various clinical trials.

Research Directions and Anticipated Needs:

The most important issues that the workshop participants expressed consist of 1) the need for natural history studies, 2) newborn screening programs, and 3) meaningful clinical outcome measures.

It is becoming clear that initiating therapy as early as possible is critical for the effective treatment of these diseases. This highlights the need for newborn screening and the collection of natural history data to move the novel therapies to the clinic.

There is growing evidence that combining various therapeutic modalities (small molecule, enzyme replacement, bone marrow transplantation, and gene therapy) can be synergistic and dramatically increase efficacy.

Over the last 5-10 years, the discussions have shifted from "how to treat these diseases" to "how do we get the therapies into the clinic". This is a very encouraging sign!