2001 Research Grants

Research Update 1

First Year Update-Canine MPS IIIB Haskins 2002

"A Proposal to Develop a Canine Model of MPS IIIB". Mark Haskins, VMD, PhD, and Matthew Ellinwood, DVM, PhD, School of Veterinary Medicine, University of Pennsylvania.

A naturally occurring canine form of MPS IIIB was identified in two Schipperke dogs, whose cases had been referred to the University of Pennsylvania. Realizing the importance of having a large animal model of this condition, we applied to The National MPS Society, and received funding to characterize the genetics and pathology of this canine model, and to establish a research colony of these dogs.

We have completed the initial characterization of the pathology seen in the first two cases of this disease and a manuscript is in preparation for submission to the Journal of Inherited Metabolic Disease. The clinical findings in canine MPS IIIB included a severe loss of balance, which began in early adulthood. Dogs also began to lose weight as the disease progressed. Both dogs were eventually euthanized because of the severe and progressive loss of balance. Analysis of these animals showed prominent GAG storage in the liver and kidneys of these dogs. The cerebellum, that part of the brain which governs balance, was severely affected, with a marked loss of neurons. Other parts of the brain showed marked neuronal storage, with storage also evident in macrophage-like cells. For the most part, the rest of the organs in the body had storage that was limited to macrophages. All tissues tested had a marked decrease of N acetyl-a-D-glucosaminidase (NAGLU) activity, to about 5% of normal. In tissues with severe storage, there were elevations seen in other enzymes, including b-glucuronidase and total b-hexosaminidase.

We have now isolated and sequenced the entire protein-coding region of the canine NAGLU gene. Our sequence extends from position -7 from the first ATG (in the context of a strong initiator site from -3 to +4), to the polyadenylation signal. The open reading frame of 2244 nucleotides bears substantial identity to human (87%) and mouse (84%) NAGLU sequences. The predicted protein comprises 747 amino acids, again with considerable identity to the human (83%) and mouse (79%) proteins. The amino terminus contains a stretch of 23 hydrophobic amino acids consistent with a signal peptide. The predicted mature protein, minus any possible carbohydrate residues, comprises 724 amino acids with a mass of 81.2 kDa. These data are due to be presented at the American Society of Human Genetics, in October of 2002.

The identification of the normal canine NAGLU sequence will be critical in developing this model as a means to evaluate enzyme replacement and gene therapies. The normal NAGLU gene sequence will allow us to next identify the disease causing mutation, and develop a DNA-based diagnostic, to be used to manage the research colony, and to help identify carrier animals in the pedigreed Schipperke population.

During the first year of this grant award we have produced two litters of pups, both sired by a

Schipperke dog that is a carrier for canine MPS IIIB. Our breeding population in the colony is now two carrier males and four carrier females. We have three pregnant females at this time, with pups due in late June and July of 2002. We expect up to 8 litters in the coming year. Pups with MPS IIIB will be monitored very closely to document all early clinical signs and evidence of disease pathology.

The preliminary characterization of this model was presented at the American Society of Human Genetics meeting in San Diego in October of 2001, with acknowledgements to The National MPS Society for funding support (Ellinwood N.M., Wang P., Skeen T., Sharp N., Cesta M., Bush W., Hardam E., Haskins M.E., Giger U. (2001) Characterization of a canine model of mucopolysaccharidosis IIIB. American Journal Of Human Genetics. 69, Suppl. 482).

Research Update 2

MPS Update Sands 2002

Engraftment of human hematopoietic cells leads to widespread distribution of donor-derived cells and correction of tissue pathology in a murine xenotransplant model of lysosomal storage disease.

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Bone marrow transplantation (BMT) is a relatively effective form of therapy for some lysosomal storage diseases. However, BMT has limitations including the harsh conditioning regimens (irradiation or cytotoxic drugs) required for engraftment and the possibility of life threatening immune reactions due to donor incompatibility. Some of the immune complications may be overcome by genetically modifying (gene therapy) the affected patient's own bone marrow cells outside the body (ex vivo). These genetically modified cells now produce the deficient enzyme and can be transplanted back into the patient where they can give rise to all of the blood cells. This gene therapy approach has proven effective in some mouse models of lysosomal storage disease. However, in those experiments mouse bone marrow cells, not human cells, are genetically modified to produce the deficient enzyme. Unfortunately, the gene therapy techniques used in the mouse studies have proven ineffective in human bone marrow cells. Therefore, alternate gene therapy techniques need to be developed and tested directly in human cells. In order to accomplish this goal we recently developed a mouse model of mucopolysaccharidosis type VII (MPS VII, b-glucuronidase deficiency) that is capable of accepting human bone marrow cells. The transplantation and engraftment of cells from one species (human) into another (mouse) is referred to as xenotransplantation. When normal human bone marrow cells are injected into the xenotransplantation model of MPS VII they repopulate the blood system, and bone marrow-derived cells can be found in most tissues of the body. The number of human cells and level of enzyme activity in the transplanted mice is sufficient to reduce or nearly eliminate the lysosomal storage in many tissues. This xenotransplantation mouse will now allow us to test new gene therapy approaches directed at human bone marrow

cells within the context of an authentic model of lysosomal storage disease. We have preliminary data showing that we can genetically modify human bone marrow cells and transplant them into the mouse xenotransplant model. The genetically modified human cells are producing a marker gene (non-therapeutic) which is easy to follow in the mouse. We have recently developed a gene therapy vector containing the b-glucuronidase gene (therapeutic) and are currently testing this in the mouse model. This type of experiment will provide important pre-clinical data before these novel approaches are attempted in affected patients.

Research Update 3

Substrate Deprivation Therapy for MPS: Iminosugar Inhibitors of Sulfotransferase

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Current therapies for the treatment of the mucopolysaccharidoses focus on methods of increasing enzyme concentration in patients to compensate for the enzyme deficit. Lowering the rate of biosynthesis of mucopolysaccharides should decrease accumulation of this substrate. The goal of any substrate deprivation therapy is not inhibit biosynthesis completely but to reduce this capability to levels commensurate with normal growth and development. We have investigated iminosugar inhibitors of 2-0-sulfotransfersases and N-deacetylase/N-sulfotransferase in mucopolysaccharide (glycosaminoglycan, GAG) biosynthesis directed towards agents for Hunter (deficient of iduronate 2-0-sulfatase) and Sanfilipo (deficient of N-acetylglucsamine N-sulfatase) syndromes. We have synthesized 34 gem-diamine 1-N-iminosugars as substrate analogs based on the structure of the constituent carbohydrates involved in heparan sulfate, one glycosaminoglycans (GAG). They resemble iduronic acid of N-acetylglucosamine, the acceptor sugar moieties acted upon by 2-0-sulfotransfersases or N-deacetylase/N-sulfotransferase, respectively. We considered conversion of functional groups and/or stereochemistry of gem-diamine 1-N-imosugars corresponding to the above acceptor sugar moieties (iduronic acid and N-acetylglucosamine) as an initial probe of structure - activity profile. These compounds contain the different functional groups and/or stereochemistry at positions of sulfation from the original sugar moieties of heparan sulfate, and are also alkylated by the various fashions at position linked to the adjacent sugar moieties in heparan sulfate for binding more avidly to the sulfotransferases. These compounds will be evaluated for inhibitory activity against heparan sulfate 2-0-sulfotransfersases and heparan sulfate N-deacetylase/N-sulfotransferase Initial studies will focus on determining the apparent Km values for the natural acceptor. The various inhibitors will then be tested at different concentrations to determine relative inhibitory constants.

Based on the biological data, we will further study on the structure-activity relationship of 1-N-iminosugars directed toward a candidate for substrate deprivation therapy of MPS.

Research Update 4

Anne K. Hennig, Ph.D., is a 2001 recipient of an MPS Society two-year research grant for her project "In Utero Gene Therapy for Mucopolysaccharidoses". Dr. Hennig works with Dr. Mark Sands at Washington University School of Medicine in St. Louis, MO. Following are the findings from the second year of her grant.

The proposal I originally submitted was for investigating "In Utero Gene Therapy for Mucopolysaccharidosis," but because of technical difficulties associated with injecting the gene therapy into mouse fetuses that project is behind schedule and I do not yet have results beyond those I shared in the last progress report.

During the funding period I was also performing another series of experiments in which an identical adeno-associated virus (AAV) gene therapy vector was injected into the vitreous humor in the eyes of young adult MPS VII mice. These mice have no functional beta-glucuronidase (GUSB) and model Sly Syndrome. The objective of the study was to determine whether reducing lysosomal storage within the eye prevented retinal degeneration, and investigate what effect the treatment had on retinal function. We found high levels of GUSB enzyme expression within treated retinas 4 to 6 weeks after treatment, and the enzyme levels were still high 12 weeks after treatment, the endpoint of the study. Interestingly, GUSB activity also appeared in the optic nerve and the parts of the brain where axons from the injected eye travel. To try to determine whether this enzyme activity was being produced locally by brain cells that had been infected by the gene therapy viral vector, I looked for genetic material from the virus in small tissue samples from different sites showing enzyme activity. The PCR assay I used could detect a single virus genome in one cell among 2 -10 thousand uninfected cells. Testing tissue samples from three treated mice, I found evidence of viral DNA in the injected eyes but not in the optic nerves coming from those eyes, or in any of the brain regions that contained GUSB activity. This indicated that the GUSB enzyme in the brain was probably not being made by the brain cells in the areas where I saw the enzyme activity. Therefore, GUSB was most likely produced in the treated eye and transported along the optic nerve into the brain.

Brains from some of the treated animals were examined for lysosomal storage. Storage was reduced in the parts of the brain along the visual pathway where we had seen GUSB activity, and also in other visual areas farther away. This indicated that small amounts of enzyme, too little to detect with our staining assay, must have been being passed along the next set of connections in the visual pathway. What surprised us was that correction of lysosomal storage was also seen in other parts of the brain that were not involved in vision but happened to be located next to visual areas. The most exciting of these areas was the hippocampus, which stores and recalls memories as part of the learning process. Unfortunately, our treated MPS VII mice could not be given behavioral tests because their systemic disease had progressed too far (we only treated their eyes). It will be important to determine whether cognitive function might be restored by this approach.

A manuscript describing these findings has been accepted for publication in the Journal of Neuroscience later this month. In future experiments I am planning to extend these findings to MPS IIIB knockout mice. Since these mice have lysosomal storage pathology in the eye and brain without debilitating systemic problems, I believe that behavioral testing will be possible in order to investigate the impact of the treatment on learning and memory.