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Effect of neonatal gene therapy on lumbar spine disease in mucopolysaccharidosis VII dogs

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ABSTRACT

Mucopolysaccharidosis VII (MPS VII) is due to deficient β-glucuronidase (GUSB) activity, which leads to accumulation of chondroitin, heparan, and dermatan sulfate glycosaminoglycans in various tissues including those of the spine. Associated spine disease can be due to abnormalities in the vertebrae, the intervertebral disks, or other spine tissues. The goal of this study was to determine if neonatal gene therapy could prevent lumbar spine disease in MPS VII dogs. MPS VII dogs were injected intravenously with a retroviral vector (RV) expressing canine GUSB at 2 to 3 days after birth, which resulted in transduction of hepatocytes that secreted GUSB into blood. Expression was stable for up to 11 years, and mean survival was increased from 0.4 years in untreated dogs to 6.1 years in treated dogs. Despite a profound positive clinical effect, 6-month-old RV-treated MPS VII dogs still had hypoplastic ventral epiphyses with reduced calcification in the lumbar spine, which resulted in a reduced stiffness and increased range of motion that were not improved relative to untreated MPS VII dogs. At six to 11 years of age, ventral vertebrae remained hypoplastic in RV-treated MPS VII dogs, and there was desiccation of the nucleus pulposus in some disks. Histochemical staining demonstrated that disks did not have detectable GUSB activity despite high serum GUSB activity, which is likely due to poor diffusion into this relatively avascular structure. Thus, neonatal gene therapy cannot prevent lumbar spine disease in MPS VII dogs, which predicts that enzyme replacement therapy (ERT) will similarly be relatively ineffective even if started at birth.

1. Introduction

The mucopolyaccharidoses (MPS) are a subset of lysosomal storage disorders characterized by deficiencies in enzymes that contribute to the degradation of glycosaminoglycans (GAGs) [1]. Disease manifestations can occur in the skeleton, liver, cardiorespiratory system, eyes, ears, central nervous system, and other sites, although the severity in different tissues varies with the specific type of MPS. The pathogenesis may involve binding of GAGs to the TLR4 and/or components of the complement cascade, resulting in induction of inflammatory cytokines and destructive enzymes [2,3].

Major manifestations of most types of MPS are skeletal abnormalities, collectively referred to as dysostosis multiplex, which are prevalent and adversely impact the patient's quality of life [4–6]. Odontoid hypoplasia can occur in the cervical spine and is associated with neck instability. The superior–anterior aspect of lumbar and thoracic vertebrae can be hypoplastic, resulting in the collapse of a vertebral body anteriorly to create a wedge-shaped vertebrae that may be associated with deformity such as kyphosis (or gibbus), and/or scoliosis. Intervertebral disks can degenerate leading to nerve root or spinal cord compression. Finally, thickening of ligaments, dura, and bony structures can also contribute to spinal cord compression [7–12]. Spine disease that is severe enough to require surgery occurs in ~10% of patients with the severe form of MPS I (Hurler syndrome) at a median age of 4 years, ~15% of patients with intermediate-severity MPS I at a median age of 13 years, and ~15% of attenuated-severity MPS I at a median age of 21 years [13]. The apparent lower incidence in patients with more severe disease likely reflects their reduced survival.

MPS VII (Sly syndrome) is due to deficient β-glucuronidase (GUSB) activity, which leads to accumulation of chondroitin, heparan, and dermatan sulfate GAGs [9,10]. Although data on the frequency of spinal disease in patients with MPS VII are not available due to the low incidence of MPS VII (~1:1,000,000), gibbus deformities have been reported [8,9]. The naturally-occurring MPS VII dog has a missense mutation (R166H) in the GUSB gene and exhibits many of the musculoskeletal manifestations of the disorder observed in humans [14–17]. In recent studies, we demonstrated that the lumbar vertebrae

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of 6-month-old MPS VII dogs had radiolucent cartilaginous lesions with reduced calcium and increased GAG content in the ventral and dorsal regions, suggestive of a failure to convert cartilage to bone during development [18]. This was likely responsible for the reduced stiffness and increased range of motion found in lumbar spine segments from MPS VII dogs [18]. Additionally, the disk annulus fibrosus (AF) contained elevated levels of GAGs compared to those in normal animals [18].

Treatments that are currently used clinically for MPS include hematopoietic stem cell transplantation (HSCT) and enzyme replacement therapy (ERT), while gene therapy is being investigated in animal models. The efficacy of HSCT is likely due in large part to migration of enzyme-sufficient normal blood-derived cells into tissues, which secrete enzyme with mannose 6-phosphate (M6P) locally that can be taken up by nearby cells via the M6P receptor, although secretion of enzyme into blood could also play a role. HSCT has not prevented spine disease [19], which may be because of the relatively avascular nature of many spine components and the paucity of blood-derived cells. ERT is only available for certain types of MPS and involves intravenous injection (IV) of M6P-modified enzyme into blood, which can diffuse to other tissues and be taken up via the M6P receptor. The effect of ERT on spine disease is somewhat difficult to assess, as the first drug was only approved in 2003, and many of the patients had manifestations of skeletal disease at the time of therapy initiation. Some have reported that spine stability is adversely affected in MPS by ERT, which may relate to clearance of GAGs from soft tissues, allowing structural instability from ligament and bone abnormalities to manifest [20]. One gene therapy approach involved intravenous (IV) injection of a gamma retroviral vector (RV) expressing canine GUSB to newborn MPS VII animals, which led to transduction of liver cells and secretion of M6P-modified GUSB into blood, from where enzyme could diffuse to other organs and be taken up via the M6P receptor [21]. Gene therapy has shown promise in correcting some musculoskeletal manifestations of MPS, as RV-treated MPS VII mice and/or dogs have improved growth plate morphology, partially normalized lengths of bones including the vertebral bodies, improved facial morphology, and reduced erosions of femoral heads [22,23]. However, the effect of gene therapy on the biomechanical function of the spine has not been addressed. The objective of this study was to determine if neonatal RV-mediated gene therapy of MPS VII dogs could reduce lumbar spine disease.

2. Methods

2.1. Animals and sample preparation

The animals used in this study were raised at the School of Veterinary Medicine at the University of Pennsylvania, under NIH and USDA guidelines for the care and use of animals in research. At the start of the project, dogs that were heterozygous for MPS VII ($GUSB^{+/-}$) were bred to generate affected $GUSB^{-/-}$, heterozygous $GUSB^{+/-}$, or homozygous $GUSB^{+/+}$ dogs that were identified by PCR of blood cell DNA at birth and confirmed with GUSB enzyme assay of serum. The gamma Moloney murine leukemia-based LNL6 vector designated hAAT-cGUSB-WPRE contained an intact LTR with the canine GUSB cDNA downstream of the human α_1-antitrypsin promoter [21,24]. It was packaged in the amphotropic GP+AM12 cells and concentrated by ultrafiltration followed by gel filtration to remove low molecular weight contaminants as reported previously [24]. The vector was titrated by performing GUSB staining on GUSB-deficient murine fibroblast-derived 3521 cells that were transduced after serial dilution of the vector. Some MPS VII dogs were injected IV at 2 to 3 days of age with 3 to 10×10^9 transducing units (TU)/kg of hAAT-cGUSB-WPRE and are designated as RV-treated dogs. One MPS VII dog (M1287) received hepatocyte growth factor (HGF) prior to injection of a higher dose of RV (2×10^{10} TU/kg) as detailed previously [21], and is designated as the HGF/RV-treated dog. Later, RV-treated $GUSB^{-/-}$ males were bred with heterozygous females to generate affected MPS VII and heterozygous unaffected dogs. Normal dogs at 6 months-of-age or earlier included both homozygous $GUSB^{+/+}$ and heterozygous $GUSB^{+/-}$ animals, that were all phenotypically normal. Most unaffected controls were littermates of MPS VII dogs or had at least one parent in common. Older normal dogs were heterozygous $GUSB^{+/-}$ females that had been used for breeding. Radiographs were obtained while under anesthesia with intramuscular injection of 0.02 mg/kg of atropine (Phoenix Pharmaceutical, St. Joseph MO) and 0.1 mg/kg of hydromorphone (Elkins-Sinn, Cherry Hill NJ), and IV injection of 2 mg/kg of propofol (Abbott, Chicago IL). Euthanasia was performed for animals with substantial clinical manifestations, or for collection of tissues, using 80 mg/kg of sodium pentobarbital (Veterinary Laboratories, Lenexa, KS) in accordance with American Veterinary Medical Association guidelines. Kaplan–Meier log-rank survival analysis was performed for untreated and RV-treated MPS VII dogs that were born between June of 2000 and June of 2010.

2.2. GUSB assay

Serum GUSB activity was measured using 4-methylumbelliferyl (4-MU)-β-D-glucuronide substrate and determination of the fluorescent product 4-MU using a fluorometer, as described previously [21]. Extracts from the annulus fibrosus (AF) were prepared by homogenization of samples as described [25], and GUSB activity was normalized to the amount of protein in the sample. Enzyme activity of 1 unit (U) indicates conversion of 1 nmol of substrate to product in 1 h at 37 °C. Eight micrometer-thick frozen sections were stained for GUSB activity using naphthol AS-BI-β-D-glucuronide as described [21].

2.3. Histology and biomechanics

Lumbar spines (T12-sacrum) were dissected out immediately following euthanasia and frozen. L2–L3 spine segments were thawed, fixed for 1 week in buffered 10% formalin, then decalcified for 1–2 weeks in formic acid/EDTA (Formical-2000; Decal Chemical Corporation, Tallman, NY). A 5 mm thick, mid-sagittal slab was processed into paraffin and 10-μm-thick sections were double-stained with alcian blue and picrosirius red to demonstrate GAG and collagen, respectively [18]. Histology was undertaken for 6-month old normal, untreated MPS VII, and RV-treated MPS VII animals, and for older normal and RV-treated MPS VII animals.

L1–L2 spine segments (vertebra–disk–vertebra) were thawed and lateral radiographs taken using a fluoroscope (Hologic, Bedford, MA, USA). Biomechanical testing was carried out according to techniques published previously by our laboratory [18]. Briefly, following overnight equilibration in PBS, samples were tested in 20 tension–compression loading cycles between 45 and −90 Newtons (N) (Instron, Norwood, MA, USA) at a strain rate of 0.3 mm/s. Compressive and neutral zone stiffness (N/mm) and range of motion (mm) were calculated as described previously [18]. Biomechanical testing was performed for 3 RV-treated dogs at 6 months of age, and was compared with those for age-matched normal and untreated MPS VII dogs that were previously evaluated using the same personnel and equipment [18].

2.4. GAG, water, and calcium contents

Samples were obtained from whole nucleus pulposus (NP) and outer ventral AF by sharp dissection of the L5–L6 disk and by bone shears to obtain cortical and trabecular bone of the ventral epiphysis of the L6 vertebral body. Epiphysis samples were divided into two equal portions and pulverized. Specimens were weighed to determine wet weight, dried overnight at 60 °C, and re-weighed to determine dry weight and calculate the water content. NP and AF samples and half of the epiphysis were then digested overnight at 56 °C using proteinase K. Sulfated GAG was measured using the dimethylmethylene blue assay [26]. The second half of the epiphysis was incubated overnight in 0.5 M