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Diffusion tensor imaging and myelin composition analysis reveal abnormal myelination in corpus callosum of canine mucopolysaccharidosis I

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ABSTRACT

Children with mucopolysaccharidosis I (MPS I) develop hyperintense white matter foci on T2-weighted brain magnetic resonance (MR) imaging that are associated clinically with cognitive impairment. We report here a diffusion tensor imaging (DTI) and tissue evaluation of white matter in a canine model of MPS I. We found that two DTI parameters, fractional anisotropy (a measure of white matter integrity) and radial diffusivity (which reflects degree of myelination) were abnormal in the corpus callosum of MPS I dogs compared to carrier controls. Tissue studies of the corpus callosum showed reduced expression of myelin-related genes and an abnormal composition of myelin in MPS I dogs. We treated MPS I dogs with recombinant alpha-L-iduronidase, which is the enzyme that is deficient in MPS I disease. The recombinant alpha-L-iduronidase was administered by intrathecal injection into the cisterna magna. Treated dogs showed partial correction of corpus callosum myelination. Our findings suggest that abnormal myelination occurs in the canine MPS I brain, that it may underlie clinically-relevant brain imaging findings in human MPS I patients, and that it may respond to treatment.

1. Introduction

MPS I (a.k.a. "Hurler syndrome") is an inherited disease that causes progressive loss of cognitive function and substantial physical disease in children. In MPS I, glycosaminoglycans accumulate intracellularly, due to the deficiency of the lysosomal enzyme alpha-L-iduronidase. However, glycosaminoglycans are not directly toxic, and the cause of neurological deterioration in children with MPS I is not presently clear. Brain histological findings in MPS I patients have shown neuronal cell loss, gliosis, swelling of cell bodies and dendrites, prominent perivascular (i.e., Virchow–Robin) spaces, leptomeningeal thickening, and gross atrophy (Naidoo, 1953). In addition, brain MR imaging findings show hydrocephalus, cribriform changes, and hyperintense lesions of white matter (Seto et al., 2001). Some investigators have speculated that the hyperintense lesions of white matter are caused by abnormal myelination (Gabrielli et al., 2004). In this study, we performed a controlled, preclinical study of MPS I and carrier control dogs to determine whether white matter abnormalities described in human MPS I could be detected in MPS I dogs. Canine studies are essential because humans affected with severe MPS I disease typically receive bone marrow transplantation and immune suppression therapy, which could confound observational imaging studies and studies of novel therapies.

In previous studies, we used a naturally-occurring canine model of MPS type I to study the neurological disease due to MPS I and its potential treatment (Dierenfeld et al., 2010; Shull et al., 1982; Vite et al., 2013). In one study, we used MR imaging to assess anatomic and structural features of the brains of three canine populations: nine adult MPS I dogs, four age-matched, unaffected carrier dogs and four MPS I dogs that had been treated beginning at four months of age with intrathecal

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recombinant human alpha-L-iduronidase at three month intervals (Vite et al., 2013). The study showed that the canine MPS I brain shares many anatomic features with human MPS I disease including ventriculomegaly, brain cortical atrophy, and volume loss in the corpus callosum that was prevented by treatment (Vite et al., 2013). The neuroimaging findings of volume loss in the corpus callosum (a major white matter structure) and hyperintense regions within the white matter suggested the possibility of white matter involvement, including demyelination. With this in mind, we set out to study brain microstructure in MPS I dogs using MR techniques that would reflect myelination changes on the microscopic level and correlate those findings with the composition of myelin and expression of myelin-related genes.

DTI is a MR technique that provides information about the microstructure of white matter through measurement of the microscopic, three-dimensional motion of water. DTI studies of the brain in MPS I children have shown reduced fractional anisotropy (FA) in the corpus callosum, a finding that has been correlated with reduced attention and suggests that abnormalities within white matter may underlie some aspects of the loss of function in MPS I patients (Shapiro et al., 2012). Another pertinent DTI metric is radial diffusivity, which has been shown to correlate with the absence of myelination (Song et al., 2002).

In the present study, we set out to correlate DTI findings and features of demyelination of the same dogs that were examined in our previous study. Our first hypothesis was that both FA values and RD values would be altered in the white matter of untreated MPS I dogs. Our second hypothesis was that these findings would correlate with degree of myelination (as measured by levels of myelin basic protein (MBP) as well as altered myelin composition and diminished expression of myelin-related genes). Our third hypothesis was that treatment with intrathecal recombinant human alpha-L-iduronidase would ameliorate both the altered DTI metrics and the pre-existent defects in myelin composition and gene expression. Finding of a correlation between DTI parameters and a quantitative measurement of degree of myelination is very important if DTI is to be useful as a means to measure therapeutic response in demyelinating diseases. As a representative white matter structure, we chose the corpus callosum, a highly anisotropic structure that is easily identified in the canine brain.

2. Materials and methods

2.1. Study design

2.1.1. Research objectives

We performed a controlled, preclinical study of MPS I and carrier control dogs to determine whether white matter abnormalities described in human MPS I could be detected in MPS I dogs. Canine studies were essential, because patients affected with severe MPS I disease typically receive bone marrow transplantation and immune suppression which could confound observational imaging studies in human subjects. Following our initial determination of reduced fractional anisotropy in the corpus callosum of affected dogs vs. controls, we performed further data and tissue analyses to determine whether the changes reflected abnormal myelination. We also studied treated MPS I dogs to determine whether the imaging and compositional abnormalities that we discovered would respond to therapeutic intervention.

2.1.2. Research subjects

Dogs were bred from the original Plott hound colony (Shull et al., 1982). Study subjects were maintained in accordance with US Department of Agriculture and NIH guidelines for the care of dogs. All study procedures were reviewed and approved by institutional animal care and use committees. Dogs were bred and maintained as described (Vite et al., 2013). We used a mix of heterozygous and homozygous normal control dogs from this kindred. Heterozygous dogs are carriers only and have never been documented to have manifestations of MPS I disease at any time. The dogs in this study were previously described with respect to their brain magnetic resonance imaging findings (Vite et al., 2013).

2.1.3. Experimental design

Treated dogs (both genders) received recombinant human alpha-L-iduronidase (formulated as laronidase, BioMarin Pharmaceutical, Novato, CA). For intrathecal treatment, 0.05 mg/kg body weight (up to 1 mg) recombinant human alpha-L-iduronidase was diluted in 1:2 (v/v) Elliotts B artificial spinal fluid (DRAXIS Pharma, Kirkland, Quebec, Canada) and administered into the cisterna magna at three month intervals as previously described (Vite et al., 2013). Four of the nine untreated MPS I dogs (I-371, I-388, I-392 and I-393) received intra-articular treatment with 1 mg recombinant human alpha-L-iduronidase to the right stifle (knee) and elbow joints once monthly for six months in a separate study (Wang et al., 2014). Intra-articular treatment would not be expected to affect MPS disease in the brain. Dogs received 2.2 mg/kg diphenhydramine prior to intrathecal recombinant human alpha-L-iduronidase administration to prevent infusion reactions.

2.1.4. Sample size

We used heterozygous normal control dogs from this kindred for this study. Heterozygous dogs are carriers only and are not expected, and have never been documented in the over 30 years that the model has existed at five different sites, to have manifestations of MPS I disease at any time. Animals of this kindred are preferred controls relative to other wild-type dogs from outside of the colony (which could have other genetic variations). Sample size was determined mainly by availability of animals. However, we estimated that there is a 25% reduction in fractional anisotropy of the corpus callosum in human MPS I patients compared to controls. Power analysis suggests that four animals per group would yield >97% power to detect a 25% change with an estimated SD of 10%.

2.1.5. Data inclusion and outliers

No animals were lost during the study, and data from all animals were included. No data were treated as outliers. Two neuroimaging studies with high levels of artifact (such as motion artifact) were identified, not analyzed, and excluded from the data set. Since with one exception at least two and in some cases three scans were performed for each animal, we were able to include imaging studies for each dog in the results. More information about excluded imaging studies can be found below under "DTI."

Table 1
Canine subjects used in the study. The "X" marks studies in which a particular dog was included. MPS: mucopolysaccharidosis; MTr: MPS I dogs treated with intrathecal recombinant human alpha-L-iduronidase; DTI: diffusion tensor imaging; RT-PCR: reverse transcriptase-polymerase chain reaction. Dogs that received intra-articular alpha-L-iduronidase are marked with an *.

Subject	Group	DTI	RT-PCR	Myelin composition	Age at necropsy
I-319	CTL	X	X	X	19 m
I-318	CTL	X	X	X	19 m
B-301	CTL	X	X	X	30 m
I-262	CTL	X	X	X	29 m
I-371*	MPS	X	X		20 m
I-388*	MPS	X	X		17 m
I-392*	MPS	X	X		17 m
I-393*	MPS	X	X		17 m
I-399	MPS	X			21 m
I-272	MPS	X	X	X	25 m
I-266	MPS	X	X	X	25 m
I-267	MPS	X	X	X	26 m
I-265	MPS	X	X	X	26 m
I-394	MTr	X	X	X	21 m
I-400	MTr	X	X	X	21 m
I-402	MTr	X	X	X	21 m
I-413	MTr	X	X	X	21 m