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Development of cerebellar pathology in the canine model of mucopolysaccharidosis type IIIA (MPS IIIA)

Sofia Hassiotis [a,*], Robert D. Jolly [b], Kim M. Hemsley [a]

[a] Lysosomal Diseases Research Unit, South Australian Health and Medical Research Institute, PO Box 11060, Adelaide, South Australia 5001, Australia
[b] Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North 4442, New Zealand

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ABSTRACT

The temporal relationship between the onset of clinical signs in the mucopolysaccharidosis type IIIA (MPS IIIA) Huntaway dog model and cerebellar pathology has not been described. Here we sought to characterize the accumulation of primary (heparan sulfate) and secondary (G_{M3}) substrates and onset of other changes in cerebellar tissues, and investigate the relationship to the onset of motor dysfunction in these animals. We observed that Purkinje cells were present in dogs aged up to and including 30.9 months, however by 40.9 months of age only ~12% remained, coincident with the onset of clinical signs. Primary and secondary substrate accumulation and inflammation were detected as early as 2.2 months and axonal spheroids were observed from 4.3 months in the deep cerebellar nuclei and later (11.6 months) in cerebellar white matter tracts. Degenerating neurons and apoptotic cells were not observed at any time. Our findings suggest that cell autonomous mechanisms may contribute to Purkinje cell death in the MPS IIIA dog.

1. Introduction

Mucopolysaccharidosis type IIIA (MPS IIIA; MIM 252900) is a neurodegenerative lysosomal storage disorder (LSD) characterized by a deficiency in N-sulfoglucosamine sulfohydrolase (SGSH; EC 3.10.1.1), a lysosomal enzyme required to catabolize the glycosaminoglycan, heparan sulfate [1]. SGSH deficiency causes primary storage of partially-degraded heparan sulfate, the accumulation of which may inhibit other lysosomal enzymes [2], potentially leading to secondary accumulation of gangliosides G_{M2} and G_{M3}. The principal site of pathology is the central nervous system. Clinical symptoms include speech delay, sleep disturbance, hyperactivity, aggression and anxiety, with progressive neurological and behavioral deterioration and early death [3]. The Australian clinical prevalence of MPS IIIA is 1/114,000 live births [4] and no treatment is currently available.

The MPS IIIA Huntaway dog is a naturally-occurring large animal model of the human disease, in which the clinical signs are predominantly those of a movement disorder, developing from approximately 24 months of age and attributed to lesions in the cerebellum [5,6]. It results from an insertion mutation of adenosine at amino acid position 708–709, leading to a frameshift and early termination of SGSH at position 228 [7]. There is no detectable SGSH activity [8], with consequent accumulation of heparan sulfate and secondary substrates such as gangliosides [9]. MPS IIIA dogs are often euthanized early because of the progressive nature of clinical signs, which include ataxia, hypermetria and wide-base stance of limbs. Intention tremor is not observed.

Description of the nature of cerebellar lesions in the dogs is mainly limited to late-stage disease, including accumulation of storage cytosomes in Purkinje cells (PKC) as well as PKC loss [6]. Storage cytosomes are complex and ultrastructurally, appear mostly comprised of membranous materials (i.e. lipids), with accumulation of granular material (postulated to be precipitated glycosaminoglycan) observed in macrophages and visceral organs [6]. The storage cytosomes have an orange/yellow autofluorescence as well as variable staining characteristics with general histological stains such as Sudan black, periodic acid Schiff and luxol fast blue [5,6]. Mild to moderate loss of granule cells, mild proliferation of Bergmann glia including hypertrophy of Bergmann glial processes and the occasional spheroid in the cerebellar white matter tracts have also been reported [6]. Neuroinflammation has been observed in the cerebellum and other brain regions [9].

PKC are the sole source of cerebellar signaling and their loss has been implicated in cerebellar dysfunction in other animal models of LSD, e.g.

Abbreviations: $GAD_{65/67}$, glutamic acid decarboxylase 65/67; GlcNS-UA, glucosamine-N-sulfate-uronic acid; INCL, infantile neuronal ceroid lipofuscinosis; LC–ESI-MS/MS, liquid chromatography–electrospray ionization tandem mass spectrometry; LSD, lysosomal storage disorder; MPS IIIA, mucopolysaccharidosis type IIIA; MPS IIIB, mucopolysaccharidosis type IIIB; NP, Niemann–Pick; PKC, Purkinje cell(s); RCA-1, ricinus communis agglutinin-1; rTdT, recombinant terminal deoxynucleotidyl transferase; SGSH, N-sulfoglucosamine sulfohydrolase; TUNEL, terminal dUTP nick end labeling.

* Corresponding author at: Research Scientist, CNS Therapeutics Lab, Lysosomal Diseases Research Unit, PO Box 11060, Adelaide, South Australia 5001, Australia.
E-mail addresses: Sofia.Hassiotis@sahmri.com (S. Hassiotis), R.D.Jolly@massey.ac.nz (R.D. Jolly), Kim.Hemsley@sahmri.com (K.M. Hemsley).

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canine mucopolysaccharidosis type IIIB (MPS IIIB) [10] and fucosidosis [11,12], as well as α-mannosidosis guinea pigs [13] and murine models of MPS IIIB [14], infantile neuronal ceroid lipofuscinosis (INCL) [15] and Niemann–Pick (NP) types A, B and C [16–19]. In α-mannosidosis guinea pigs and some of the murine models, the loss of PKC occurs in an anterior to posterior direction, correlating with a loss of calbindin-D28K, a calcium-binding protein involved in calcium homeostasis [13,15,16,18,19]. Whilst calcium homeostasis has not been examined in the MPS IIIA dog, dilated stacks of smooth endoplasmic reticulum have been noted in hypertrophied PKC [6]. As the endoplasmic reticulum is a site of calcium ion storage, this suggests perturbed calcium flux [6].

Using both histological and biochemical methods, this retrospective study on archival material examined the development of cerebellar lesions in relation to the onset of clinical signs in the canine model of MPS IIIA.

2. Materials and methods

2.1. Animals and tissue collection

Archival material obtained from unaffected (heterozygous) and affected (MPS IIIA) dogs bred and housed in a research kennel facility, was used in this study. All dogs had been genotyped using published methods [7] and had undergone routine neurological examinations (personal communication Dr Neil Marshall). Tissues were collected for studies approved by the Animal Ethics Committee of Massey University (Palmerston North, New Zealand) and adhered to 'The Code of Ethical Conduct for the Use of Live Animals for Teaching and Research' (New Zealand 'Animal Welfare Act 1999'). Approval was also obtained by the Animal Ethics Committee of the Children, Youth and Women's Health Service (Adelaide) and adhered to the 'Australian Code of Practice for the Care and Use of Animals for Scientific Purposes', 7th edition, 2004.

At the time of euthanasia most of the animals' brain (unaffected n = 6; MPS IIIA n = 10) and cerebellar (unaffected n = 2; MPS IIIA n = 7) weights were recorded. The brain was then bisected along the sagittal midline, producing a left and right hemi-cerebellum. Samples available for the study included fresh frozen tissue from the cerebellar mid-line dorsal lobules 4/5 of the right hemi-cerebellum and formalin-fixed, paraffin-embedded or snap-frozen tissue from sagittal mid-line, medial or lateral left hemi-cerebellum. Unaffected and MPS IIIA animals were then grouped according to age and genotype and on the basis of the outcome of neurological exams carried out prior to euthanasia, the MPS IIIA group was further divided into a 'pre-symptomatic' cohort (where no clinical signs referable to the disease were observed) or a 'symptomatic' group (where clinical signs of ataxia, hypermetria and wide-based stance were present). The groups are outlined in Table 1.

Table 1
Animal groups and number of cerebellar lobules available for the study. F = female; M = male; a = frozen sections of posterior lobe of cerebellum available for G_{M3} immunohistochemistry.

Genotype & gender		Age (months)	Total (n)	Presence of cerebellar lobules									
				1	2	3	4	5	6	7	8	9	10
Unaffected			6										
M		2.2[a]			●	●	●	●	●	●			
M		3.6[a]			●	●	●	●	●	●	●	●	
F		33.0				●	●	●	●	●	●	●	●
F		33.0						●	●	●	●	●	●
F		39.0		●	●	●	●	●	●	●	●	●	●
F		67.5[a]							●	●	●	●	
MPS IIIA			10										
Pre-symptomatic	M	2.2[a]						●	●	●	●		
(<12 months)	F	4.3		●	●	●	●	●	●	●	●	●	●
	F	6.0[a]					●	●	●	●	●	●	●
	F	6.0[a]				●	●	●	●	●	●	●	
	M	6.0[a]			●	●	●	●	●	●	●		
	M	11.6[a]				●	●	●	●	●	●	●	●
Symptomatic	M	30.9[a]				●	●	●	●	●	●	●	
(>30 months)	F	40.9[a]							●	●	●	●	
	F	43.7							●	●	●		
	F	46.9				●	●	●	●	●	●	●	●

2.2. Reagents for staining

Mouse monoclonal antibodies to ganglioside G_{M3} (Clone GMR6; #370695) and calbindin-D28K (#C9848) were purchased from Seikagaku Corporation (Tokyo, Japan) and Sigma-Aldrich (St. Louis, MO, USA), respectively. Polyclonal rabbit antibodies to ubiquitin (#Z0458) and glutamic acid decarboxylase 65/67 ($GAD_{65/67}$; #G5163) were obtained from DAKO (Glostrup, Denmark) and Sigma-Aldrich (St. Louis, MO, USA) respectively. A biotinylated lectin from ricinus communis agglutinin 1 (RCA-1; #b1085) was purchased from Vector Laboratories (Burlingame, CA, USA). FluoroJade B (#AG310) was purchased from Millipore (Temecula, CA, USA). Recombinant terminal deoxynucleotidyl transferase (rTdT; #M1875) and biotin-16-dUTP (#11093070910) for the terminal dUTP nick end-labeling (TUNEL) assay were obtained from Promega Corporation (Wisconsin, USA) and Roche Diagnostics (Mannheim, Germany), respectively. Biotinylated donkey anti-rabbit IgG (#711-065-152) and biotinylated donkey anti-mouse IgG (#715-065-150) secondary antibodies were purchased from Jackson Immunoresearch Laboratories (West Grove, PA, USA). Biotinylated goat anti-mouse IgM (#BA2020) was obtained from Vector Laboratories (Burlingame, CA, USA).

2.3. Immunohistochemistry and histochemical methods

Six-micron thick paraffin sections of sagittal cerebellum, cut using a rotary Leica microtome (RM2235), were stained in batches. Briefly, for immunohistochemistry, sections were de-waxed and rehydrated prior to pre-treatment with 0.05% trypsin (Sigma; #T8128) for calbindin-D28K. Pre-treatment was not required for ubiquitin and $GAD_{65/67}$. Endogenous peroxidases were quenched with 3% hydrogen peroxide; following blocking of non-specific proteins with 10% normal serum (specific to the secondary antibody), primary antibodies (1/1000 calbindin-D28K; 1/8000 ubiquitin; and 1/4000 $GAD_{65/67}$) were incubated overnight at room temperature. Sections were then incubated with the appropriate biotinylated secondary antibody, donkey anti-mouse IgG (1/800) or donkey anti-rabbit (1/800). To visualize staining, sections were then conjugated with avidin (Vector ABC Elite kit; PK6100; Vector Laboratories) and developed using a DAKO liquid DAB chromogen kit (DAKO; #K346811). Histochemical staining of microglia with biotinylated lectin RCA-1 was performed using a modified method from Kiatipattanasakul et al. [20], involving pre-treatment with 0.05% Trypsin and quenching of endogenous peroxidases as described above, followed by overnight incubation of biotinylated lectin RCA-1 (1/2500) with visualization as described above. The sections stained with calbindin-D28K, ubiquitin, $GAD_{65/67}$ and RCA-1 were lightly counterstained with hematoxylin.

Using a cryostat (Thermo Electron Corporation, Cheshire, UK), six-micron thick frozen sections of sagittal posterior cerebellar lobe were cut for immunohistochemical detection of G_{M3} (1/750) using published methods [21,22]. Samples available for G_{M3} staining are indicated in Table 1. Frozen tissue from one additional MPS IIIA (25 months) and two unaffected dogs (6 months) became available during the study.

The presence of neuronal degeneration in sagittal cerebellar paraffin sections was assessed by FluoroJade B staining using previously published methods [23,24]. Detection of apoptosis via TUNEL assay utilized a method modified from Gavrieli et al. [25] and Portera-Cailliau et al. [26]. Modifications made included blocking endogenous peroxidases with 1% hydrogen peroxide in 10 mM Tris buffer, pH 8.0 and using a reaction solution containing 0.03 units of rTdT and 9 nmol of biotinylated