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Injection of recombinant human sulfamidase into the CSF via the cerebellomedullary cistern in MPS IIIA mice ☆

Kim M. Hemsley [a,b,*], Barbara King [a], John J. Hopwood [a,b]

[a] *Lysosomal Diseases Research Unit, Department of Genetic Medicine, Children, Youth and Women's Health Service, Adelaide 5006, Australia*
[b] *Department of Paediatrics, University of Adelaide, Adelaide 5005, Australia*

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Abstract

At present, there is no widely available, safe and effective treatment for lysosomal storage disorders (LSD) that affect the brain. We have used a naturally occurring mouse model of mucopolysaccharidosis type IIIA (MPS IIIA) or Sanfilippo syndrome, to evaluate the effect of repeated injection of recombinant human sulfamidase (rhSGSH) into the cerebrospinal fluid via the cisterna magna (CM) on central nervous system (CNS) pathology and behavioral function. Mice received up to seven injections of rhSGSH (5–20 μg rhSGSH per injection) or vehicle on a fortnightly or monthly basis. A dose-dependent reduction in the level of a heparan sulfate-derived monosulfated disaccharide was observed within the brain (up to 62% reduction compared with vehicle-treated MPS IIIA mice) and spinal cord (up to 71% reduction). Ultrastructural examination revealed a reduction in lysosomal vesicle formation in various cell types and fewer (ubiquitin-positive) axonal spheroids were observed in several brain regions. The biochemical changes were accompanied by improved behavior, particularly in mice-treated more frequently. A humoral immune response to rhSGSH was observed in treated animals. Intra-CM injection of lysosomal enzyme may therefore represent an immediately applicable method of treating the CNS effects of this and potentially other LSD that affect the brain.

Keywords: MPS IIIA; Lysosomal storage disorder; Mouse; CNS; Recombinant enzyme; Cerebrospinal fluid; Mass spectrometry; Neuropathology; Behavior; Humoral immune response

Introduction

Mucopolysaccharidosis type IIIA (MPS IIIA or Sanfilippo syndrome) is an inherited lysosomal storage disorder (LSD) that results from the absence or defective function of heparan-*N*-sulfatase (sulfamidase; SGSH; EC 3.10.1.1), an enzyme that cleaves glucosamine-*N*-sulfate bonds at the non-reducing end of heparan sulfate (HS) fragments [1–3]. The main feature of this disorder is central nervous system (CNS) pathology, which results in progressive neurodegeneration and subsequent mental decline and a greatly shortened lifespan (often <20 years). Clinical symptoms include hyperactivity, aggressive behaviour and sleep disturbance [4]. Somatic pathology results in hepatosplenomegaly and mild dysostosis multiplex. Whilst intravenous (i.v.) enzyme replacement therapy is proving to be a useful treatment for reducing lysosomal storage in peripheral tissues (e.g., in MPS I [5], MPS II [6], MPS VI [7], Fabry disease [8,9], and Gaucher disease [10], unless administered at supra-therapeutic doses [11], intravenously administered lysosomal enzymes cannot gain access to the brain. It

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* Corresponding author. Fax: +61 8 81617100.
E-mail address: Kim.Hemsley@adelaide.edu.au (K.M. Hemsley).

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was recently demonstrated [12] that the lysosomal enzyme β-glucuronidase is transported across the blood–brain barrier using a mannose-6-phosphate (M6P) receptor-based mechanism, but that transport is negligible after the postnatal period.

Similarly, whilst bone marrow or hematopoietic stem cell transplantation halts disease progression (i.e., stabilises IQ) in several LSD e.g., in early presenting MPS I [13,14], MPS VII [15], α-mannosidosis, and α-fucosidosis [16,17], it carries with it an enormous risk of mortality, with up to 35% of patients dying during or directly after transplantation [18]. Furthermore, it is generally believed to be ineffective for the MPS III group of disorders [19,20], for reasons which are not yet understood. Therefore at present, there is no widely available, safe and effective treatment for all LSD that affect the CNS.

A naturally occurring mouse model for MPS IIIA [21] has been identified. The mutation in the mouse SGSH gene [D31N] affects the divalent ion binding site [22] and results in 10–20% of the normal amount of SGSH protein, with 1–3% of normal SGSH activity [23]. Since this mutation enables expression of low amounts of SGSH, the response to therapies is likely to be similar to that in most patients who also commonly express low amounts of SGSH. Studies examining the pathological, behavioral and biochemical characteristics of the model [21,23–26] have revealed that it closely resembles the human condition (Fig. 1).

Membrane-enclosed storage vesicles or residual bodies containing partially digested materials are the hallmark of pathology in neural (and other) cells in murine MPS IIIA. Various cell types become involved at different stages, with a progressive increase in the number and size of storage vesicles (Fig. 1). Neuro-inflammatory and neurodegenerative changes are also observed in this and other LSD [25,27,28]. Potentially irreversible axonal spheroid formation occurs in some neurons [25], however overt cell death

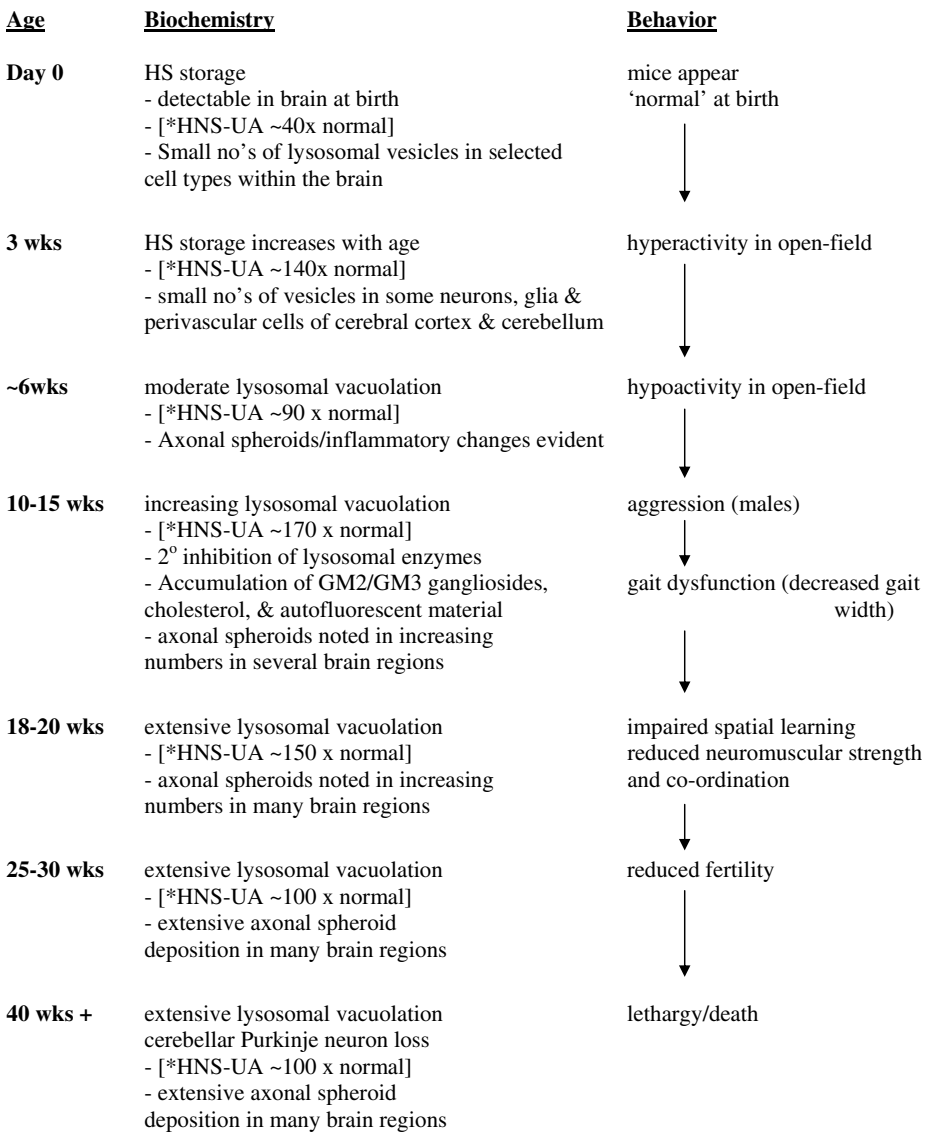

Fig. 1. Flow diagram illustrating the effect of SGSH deficiency and subsequent lysosomal storage of substrate on biochemical and behavioral parameters in the MPS IIIA mouse throughout the course of the disorder. Based on findings in [21–26,29,39].