P-Tau and Subunit c Mitochondrial ATP Synthase Accumulation in the Central Nervous System of a Woman with Hurler–Scheie Syndrome Treated with Enzyme Replacement Therapy for 12 Years

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Abstract We report an autopsy case of a woman with mucopolysaccharidosis type I (MPS I) Hurler–Scheie syndrome who was treated with enzyme replacement therapy (ERT) for 12 years. This was the first case of MPS I treated with ERT in Japan. Pathological analysis showed no glycosaminoglycan accumulation in the liver and spleen as a result of long-term ERT, although severe aortic stenosis, diffuse intimal hyperplasia of the coronary artery, and fibrous hypertrophy of the endocardium were observed. Additionally, we detected subunit c mitochon-

drial ATP synthase (SCMAS) accumulation and mild tauopathy (hyperphosphorylated tau or p-tau, both 3-repeat and 4-repeat tau accumulation) in the same area of the cerebral limbic system and central gray matter of the mid brain and pons. Tauopathy is an important pathological finding in Alzheimer's disease and other neurodegenerative disorders; however, in MPS I, it is unclear whether tauopathy is a primary or secondary phenomenon. Thus, in this report, we describe pathological accumulation of p-tau and SCMAS in the context of MPS I and discuss the mechanisms and importance of these findings in the pathogenesis of MPS I.

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Introduction

Mucopolysaccharidosis type I (MPS I) is a lysosomal storage disorder caused by mutation of the α -L-iduronidase (*IDUA*) gene, resulting in the systemic accumulation of undegraded or partially degraded mucopolysaccharides (glycosaminoglycans [GAGs]) (Neufeld and Muenzer 2001). MPS I can be divided into three clinical syndromes: Hurler syndrome (severe), Hurler–Scheie syndrome (intermediate), and Scheie syndrome (mild). Hurler syndrome presents in infancy with progressive hepatosplenomegaly, upper airway obstruction, heart valve disorder or cardiomyopathy, joint stiffness, skeletal dysplasia, corneal clouding, and neurological degeneration. Without appropriate treatment, death may occur before the first decade of life. Scheie syndrome usually presents no neurological symptoms, and

other symptoms are typically mild. Although no reports have described the mechanisms of neurological manifestation in MPS I, tauopathy and subunit c mitochondrial ATP synthase (SCMAS) accumulation have been reported in MPS IIIB and other lysosomal storage diseases (Ohmi et al. 2009).

Effective enzyme replacement therapy (ERT) for children with MPS I has been developed using recombinant human IDUA protein produced in Chinese hamster ovary cells (Kakkis et al. 1994). Systemic ERT is clinically beneficial for the non-neuropathic manifestation of MPS I, providing improvement of hepatosplenomegaly, upper airway obstruction, cardiac function, growth in height and weight, maximal range of shoulder flexion and elbow extension, and decreased urinary GAG excretion; however, this approach is not expected to be clinically beneficial for the neurological features of MPS I because of poor penetration across the blood–brain barrier (BBB) (Sohn et al. 2013). Some recent experimental trials of direct enzyme injection in MPS animal models with neuropathic symptoms have been reported. However, the effects of these therapies on the cardiac and skeletal systems are thought to be limited (Higuchi et al. 2012).

In this report, we describe a case with no neurological features, but with moderate to severe non-neuropathic manifestations, diagnosed as Hurler/Scheie syndrome and treated with systemic ERT for 12 years (Kobayashi et al. 2006). We report the results of long-term ERT for MPS type I (Hurler/Scheie syndrome) through pathological analysis performed after autopsy.

Case Report

The female patient was born with no complications (birth weight: 3,060 g). However, at 2 years of age, she displayed knock knees, abdominal distension, and MPS type I, intermediate type Hurler/Scheie syndrome, as diagnosed by IDUA enzyme assays and clinical tests. At 17 years of age, she underwent cardiac valve replacement with a mechanical valve for progressive mitral valve regurgitation. At 20 years of age, she suffered from clonus and required assistance in daily life. At 26 years of age, she exhibited cervical instability, which caused a severe cord injury, and required tracheotomy and mechanical ventilation due to respiratory failure owing to ring axis vertebral dislocation. At 29 years of age, she underwent ovariectomy due to ovarian torsion and then began to receive ERT with Aldurazyme (recombinant IDUA; Genzyme Japan) 1 year later, with weekly treatment continuing for 12 years. At 42 years of age, she died suddenly, and an autopsy was performed.

Her intelligence was not estimated by a formal IQ/DQ test, but she was able to communicate by eye contact or head movement; brain atrophy and ventricular dilatation were detected by computed tomography (CT) scanning at 30 years of age. All of her ribs were oar-shaped, as observed in the chest X-ray, and the range of motion (ROM) of each joint was restricted. After the start of ERT, uronic acid excretion in the urine (Fig. 1a) decreased by 48% within the first 52 weeks, and the volume of her liver (calculated by the field of view in abdominal CT, Fig. 1b)

Fig. 1 (a) Urine-uronic acid (Di Ferrante method). The gray area shows the normal range. (b) Time-dependent changes in liver volume (cm³, determined by abdominal CT-volumetry). The gray area shows the normal range. (c) Time-dependent changes in cardiac function,

before and after ERT, determined by echocardiogram. *EF* ejection fraction (%), *LV mass* left ventricular mass (g), *VA* aortic valvular area (cm²)

decreased to within the normal range in half a year. Her ROM also improved 1 year after the start of ERT (data not shown). Her cardiac function including ejection fraction, left ventricular mass index, and aortic valve area trace showed improvement in the first 2 years after the start of ERT but gradually deteriorated thereafter (Fig. 1c). At 12 years after the start of ERT, when she was 42 years old, she was found in cardiopulmonary arrest with bloody bowel discharge during the early morning and did not respond to resuscitation attempts. An autopsy was performed, and the cause of her death was thought to be cardiac failure due to progressive stenosis of the aorta and coronary artery with gastrointestinal bleeding.

Materials and Methods

Generation of Human Anti-Subunit c Mitochondrial ATP Synthase Antibodies

Anti-SCMAS polyclonal antibodies were raised in 4-month-old male Japanese White rabbits (Sankyo Laboratories, Tokyo, Japan) against the keyhole limpet hemocyanin (KLH) fusion synthetic peptide corresponding to N-terminal amino acids 1–19 (DIDTAAKFIGAGAATVGVC) of human SCMAS. The SCMAS peptide with Freund complete adjuvant (Pierce Biotechnology, Inc., Rockford, IL, USA) was injected subcutaneously in rabbits. Thereafter, the peptide with Freund incomplete adjuvant (Pierce Biotechnology) was injected six times at 2-week intervals. Animal sera were affinity-purified with the glutathione S-transferase-fused peptide DIDTAAKFIGA from SCMAS.

Immunohistochemistry

Immunohistochemical staining was performed as previously described (Fukuda et al. 2010). Anti-SCMAS

antibodies were used at a dilution of 1:3,000. In negative controls, the primary antibodies were omitted. We also evaluated the expression of phosphorylated tau (AT8, 1:1,000; Thermo Fisher Scientific, Tokyo, Japan), 3-repeat isoform RD4 (1E1/A6, 1:1,000; Upstate, Lake Placid, NY, USA), 4-repeat isoform RD3 (8E6/C11, 1:1,000; Upstate), and Alzheimer precursor protein A4 (APP, 22C11, 1:1,000; Chemicon, CA, USA).

Results

General Pathology

In the livers and spleens, we detected no GAG accumulation (Fig. 2), and the basic construction of each organ was not disrupted, although the thickness of the hepatic and splenic artery branches was altered (Fig. 2A-a, B-a). This result demonstrated the effects of long-term ERT. We also found no GAG accumulation in the kidney, pancreas, lung, tongue, and tracheal cartilage.

In the ileocecal mucosa, we found bleeding and white blood cell infiltration, suggesting that some inflammation was involved along with the observed gastrointestinal bleeding, which may have contributed to her death; however, the origin of the bleeding was unclear (Fig. 2c).

Her heart showed macroscopic fibrinous pericarditis and left ventricular hypertrophy, with a wall thickness of 20 mm. In the pathological study, we observed severe aortic valve stenosis with hypertrophy and sclerosis, diffuse intimal hyperplasia of the coronary artery (Fig. 3a, b), and fibrous hypertrophy of the endocardium (Fig. 3c, d). In electron microscopy, we detected zebra bodies, suggesting lysosomal accumulation in the main trunk of the right coronary artery, endocardium, and aorta (Fig. 3d–f). From these findings, we speculated that severe and progressive aortic valve stenosis induced left ventricular hypertrophy

Fig. 2 General pathology. (A) Liver tissue (hematoxylin and eosin [HE] staining, 400×). (a) Hepatic artery branch (HE, 40×); intimal thickening (arrow). (b) Liver tissue (Alcian blue staining, 40×); vacuolar degeneration (ischemic changes) occurred predominantly in

the central vein area. (B) Spleen tissue (hematoxylin and eosin [HE] staining, 200×). (a) Splenic artery branch (HE, 200×); intimal thickening (arrow). (C) Ileocecal mucosa (HE, 400×); autolysis and polymorphonuclear leukocyte infiltration (wide arrow)

Fig. 3 Cardiac system. **(a)** Stenosis of the right coronary main truncus (HE, 40 $\times$); diffuse intimal hyperplasia was observed on the inner side of the inner elastic plate (arrow). **(b)** Stenosis of the right coronary main truncus (Elastica van Gieson [EVG] staining, 40 $\times$); diffuse intimal hyperplasia was observed on the inner side of the inner elastic plate (arrow). **(c)** Fibrous hypertrophy and inflammation of the

endocardium with continuous fibrosis in the cardiac muscle (arrow) (Masson staining, 12.5 $\times$). **(d)** Zebra bodies (wide arrow) in the right coronary main truncus (EM, 30,000 $\times$). **(e)** Zebra bodies (wide arrow) in the endocardium (EM, 10,000 $\times$). **(f)** Zebra bodies (wide arrow) in the yellow spot in the thoracic aorta (EM, 10,000 $\times$)

and heart failure with coronary stenosis. These results indicate the limitation of ERT, i.e., ERT is not sufficient to reach the cardiac system.

Neuropathology

The brain weighed 875 g and showed atrophy of the gyri and enlarged ventricles. The average weight of a normal Japanese adult female brain is 1,245 g (range: 961–1,515 g); therefore, the weight of her brain was quite low. Neuronal SCMAS accumulation and zebra bodies (EM) were distributed throughout the central and peripheral nervous systems (Figs. 4a, b and 5b, c). Neurofibrillary tangles, pretangles, and neuropil threads existed in the hippocampus, parahippocampus, and central gray matter of the brain stem. These structures contained phosphorylated tau, RD3, and RD4 (Fig. 4c). We also detected many APP-immunoreactive axons in the cerebral white matter (Fig. 4c1, c2). Dislocation of the cervical vertebra caused the destruction of the spinal cord (C4–Th10) with dural thickness (Fig. 5a).

Genotyping

Genotyping by polymerase chain reaction sequencing of the *IDUA* gene from a liver specimen revealed the presence of the c.[590-2A>G;1650G>A] sequence, suggesting the occurrence of novel compound heterozygous mutations.

Discussion

MPS type I is a major disorder in Caucasians and the prototype of all MPS disorders. Recombinant IDUA (laronidase, Aldurazyme) was approved by the Food and Drug Administration (FDA) and European Agency for the Evaluation of Medical Products in 2003 (Wraith et al. 2004; Miebach 2005), and this case was the first clinical trial of ERT using recombinant IDUA in Japan. We reported that the patient had no major infusion reactions and had immunotolerance within the first 15 months after the start of therapy (Kobayashi et al. 2006; Kakavanous et al. 2003).

From this case study, we made two conclusions. First, we determined the effects and limitations of long-term ERT. As previously described, ERT using recombinant IDUA for 12 years in this case was effective for GAG reduction in the liver, spleen, kidney, pancreas, and lung, but not effective for the cardiac or central nervous systems, as shown in our pathological study. Echocardiographic findings showed temporary improvement in cardiac function by ERT, but the cardiac function gradually deteriorated from the second year. As ERT gradually became ineffective, severe, progressive aortic valve stenosis with coronary stenosis appeared to induce left ventricular hypertrophy with chronic heart failure and secondary gastrointestinal bleeding, resulting in her sudden death. These results may be partially due to the late initiation of ERT at 30 years of age. Some studies have shown that ERT has no or only minor

Fig. 4 (a) Neuronal cytoplasmic accumulation of subunit c mitochondrial ATP synthase (SCMAS) in the cerebral cortex (arrow). (b) Zebra bodies (wide arrow) in the neuronal cytoplasm (EM); black bar = 1.0 μ m. (c) Tauopathy in the parahippocampus (arrow)

(AT8, 1:1,000). (c1) Anti-tau antibody, three repeat isoform RD3 (arrow) (8E6/C11, 1:1,000). (c2) Anti-tau antibody, four repeat isoform RD4 (arrow) (1E1/A6, 1:1,000). (d) Alzheimer precursor protein A4 immunoreactive axons in the cerebral white matter

Fig. 5 (a) Destruction of the thoracic spinal cord (Th4–10) with dural thickness (C2–Th10, arrow). (b) SCMAS accumulation in the dorsal root ganglion (DRG) (arrow). (c) Zebra body in the DRG (wide arrow) (EM); black bar = 10 μ m

effects on aortic width and valve thickness once it is established, suggesting that certain treatments such as ERT cannot eliminate long-standing ventricular remodeling due to chronic alteration of gene expression in cardiac myocytes (Pasqualim et al. 2015). Recently, the effects of high-dose ERT or modified long-circulating enzyme on central nervous system or bone pathology were reported in a murine model of MPS (Ou et al. 2013; Rowan et al. 2012). In a clinical study, combination therapy of early ERT and hematopoietic stem cell transplantation, ideally before 17 months of age, is recommended to improve prognoses, including nervous and cardiac system symptoms (Whitley

et al. 1993; Kato et al. 2016). Based on this trial, additional trials of newborn screening for detecting MPS type I have started in the USA (Elliott et al. 2016). The hypothesis of lysosomal substrate accumulation due to lysosomal dysfunction resulting from the inflammatory response has been reported. Holley et al. reported that macrophage enzymes and reduced inflammation drive brain correction of MPS IIIB by stem cell gene therapy (Holley et al. 2018). In particular, they showed data indicating that treatments with an action similar to that of prednisolone may provide relief from at least some of the behavioral aspects of MPS IIIB disease. Osborn et al. reported that angiotensin receptor

blockade ameliorated MPS I cardiac and craniofacial pathology, suggesting a role for the renin angiotensin system (RAS) in MPS pathology (Osborn et al. 2017). Our case showing the existence of an inflammatory response in the pathology of some organs, such as pericardium or intestinal tract, suggests that the long-term ERT did not have an adequate effect on inflammation presumed to result from the GAG-triggered pathological cascade.

Second, in our pathological study, we found accumulation of p-tau and SCMAS in the central nervous system in our female patient with MPS I Hurler/Scheie syndrome. The recombinant IDUA enzyme cannot pass through the BBB and should therefore not be able to affect the central nervous system. Tauopathy is the general term for syndromes caused by abnormal accumulation of phosphorylated and insoluble tau protein, a microtubule binding substance. Representative diseases associated with tauopathy include Alzheimer's disease (AD), Pick disease, progressive supranuclear palsy, and corticobasal degeneration (Williams 2006). These diseases can be broadly categorized by the number of repeats in the microtubule binding area (tubulin binding repeats) in the C terminal (three repeats in Pick disease, four repeats in progressive supranuclear palsy and corticobasal degeneration, and both three and four repeats in AD). Ohmi et al. reported that p-tau protein accumulated in the medial entorhinal cortex in MPS IIIB model mice and that lysozyme protein was co-expressed in the same neurons, suggesting that MPS IIIB could be a tauopathy resembling the pathology of AD (Ohmi et al. 2009). In the other lysosomal storage disorders, such as Niemann–pick type C, Glöckner et al. reported that tau pathology induces intraneuronal cholesterol accumulation (Glöckner and Ohm 2014). In this case study of MPS I, we detected mild p-tau accumulation of AD type (both three and four repeat tau accumulation). To the best of our knowledge, there have been no reports of tauopathy in MPS I (Ginsberg et al. 1999). Because recombinant IDUA cannot pass through the BBB, the tauopathy in this case may suggest neuropathogenesis of MPS I through mechanisms similar to those observed in AD or MPS type III; however, further studies are needed to test this hypothesis. SCMAS accumulation is a major phenomenon in neuronal ceroid lipofuscinosis, and some reports have described this phenomenon in MPS type III or other lysosomal storage diseases (Anderson et al. 2013; Elleder et al. 1997; Ryazantsev et al. 2007). Kan et al. reported that delivery of an enzyme–IGFII fusion protein to the brain in mice with MPS IIIB resulted in reduction of “secondary accumulation” of p-tau, β -amyloid, Lamp-1, and SCMAS (Kan et al. 2014). Based on these different reports, there is still no definite conclusion as to whether

such protein accumulation occurs as a primary or secondary manifestation, and more case studies are required to identify the causal relationship. Additionally, the patient's genotype, with the detected compound heterozygous mutations, may have influenced her phenotype (Kwak et al. 2016).

In summary, long-term ERT for MPS I was effective. However, this case highlights the limitations of this therapy with regard to cardiac symptoms and revealed neuropathological changes, including p-tau and SCMAS accumulation, that may have led to neurodegeneration.

Compliance with Ethics Guidelines

Conflict of Interest Yoshikatsu Eto, who was one of the planners of this work, has received research grants from Sanofi K.K. and Actelion Pharmaceuticals Japan Ltd.

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Hiroshi Kobayashi, Masamichi Ariga, Yohei Sato, Masako Fujiwara, Nei Fukasawa, Takahiro Fukuda, Hiroyuki Takahashi, Masahiro Ikegami, and Motomomichi Kosuga declare that they have no conflict of interest.

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