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Pathological findings in a patient with Fabry disease who died after 2.5 years of enzyme replacement

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Abstract

We describe the postmortem findings of a 47-year-old man with Fabry disease, an X-linked glycolipid storage disorder, who was on enzyme replacement therapy with recombinant α -galactosidase A for more than 2 years. The patient had widespread atherosclerotic coronary artery disease that culminated in a massive acute myocardial infarction. Atherosclerotic lesions were seen in the right and left coronary systems, aorta, and the basilar artery. Typical Fabry cardiomyopathy and glomerular nephropathy were found. With the exception of vascular endothelial cells, extensive glycolipid storage deposits were seen in all vascular and nonvascular cells and organ systems. We conclude that, at least in this patient, repeated infusions with α -galactosidase A over a prolonged period did not appreciably clear storage material in cells other than vascular endothelial cells. These findings also illustrate accelerated atherosclerosis in susceptible patients with Fabry disease.

Keywords

Fabry disease; Lysosomes; Atherosclerosis; Myocardial infarction; Enzyme replacement therapy; Glycolipids

Introduction

Fabry disease, an X-linked systemic disorder caused by a deficiency of the lysosomal enzyme α -galactosidase A, results in a failure to metabolize α -D-galactosyl moieties [4]. This abnormality leads to the systemic deposition of glycosphingolipids, particularly globotriaosylceramide (Gb₃) [5]. In adulthood, progressive renal failure, strokes, and heart disease develop, leading to death at a median age of 50-55 years. In the past 5 years, enzyme replacement therapy (ERT) with recombinant α -galactosidase A has been added to the standard therapeutic regimen [8,17,18,22]. Targeted primarily to reverse vasculopathy of Fabry disease, ERT has been shown to markedly reduce the cellular storage deposition in vascular endothelial cells [22]. Its long-term clinical effects, however, remain to be fully assessed. No extensive pathological analysis in patients on long-term ERT has been reported thus far. We present in this report the autopsy results of a 47-year-old man who was on ERT for more than 2 years and died suddenly.

Case report

The patient was a 47-year-old man who was diagnosed with Fabry disease at age 19 with α -galactosidase A activity of 1.3% of normal controls. Since 5 years of age, he suffered from episodes of fever and acroparesthesia, hypohidrosis, poor heat and exercise tolerance, diarrhea with abdominal cramps, and bouts of depression. At 35, he developed an acute onset of right-sided weakness and difficulty with word finding. A CT scan revealed a large anterior parietal infarct, and a left carotid angiogram showed marked narrowing of mid-sylvian branches of the left middle cerebral artery (MCA) with an area of avascularity in the left anterior parietal lobe. He was put on oral warfarin therapy with no apparent stroke recurrence. His most recent head MRI scan showed old left encephalomalacia in the left frontal lobe along with white matter hyperintense lesions on T2 and fluid-attenuated inverse recovery (FLAIR) images on the left.

He had no hypertension. His renal function was normal until age 37 when his serum creatinine was found to be 1.4 mg/dL (normal 0.9-1.4) with a creatinine clearance of 78 mL/min. He had mild-to-moderate proteinuria with variable 24-h protein excretion of up to 1.2 g/24 h. At age 43, his serum creatinine level was 1.5 mg%. His total blood cholesterol at age 20 was 189 mg/dL. At age 36, it was 201 mg/dL, and his HDL and triglyceride levels were 87 mg/dL and 92 mg/dL, respectively. At age 43, total cholesterol was 241 mg/dL, LDL level was 161 mg/dL, and HDL level was 24 mg/dL.

Other abnormalities noted were bilateral pedal edema, mild-to-moderate bilateral high-frequency hearing loss, sinus bradycardia and left ventricular hypertrophy in the electrocardiogram, and mild mitral and tricuspid valve regurgitation.

His therapy included Coumadin and carbamazepine. In addition, he received intravenous α -galactosidase A infusions (agalsidase beta; Genzyme Corporation, Cambridge, MA) at a dosage of 1 mg/kg every 2 weeks for the last 2.5 years of his life.

He first complained of sharp chest pain at age 40, lasting about 30 s (not precipitated by exertion). He complained mostly of burning chest pain on and off in the years prior to his death. One week prior to his death, he again reported a burning discomfort on his chest, which increased with physical activity. He refused to seek medical attention and shortly thereafter was found dead.

Regarding his family history, his mother, two sisters, and a brother were affected by Fabry disease. Two of his maternal uncles died of the same disease. One died of a cardiac cause at

age 39 and another maternal uncle died of stroke at age 47. Another cousin with Fabry disease died of myocardial infarction at age 53.

Pathological findings

The postmortem interval was approximately 32 h before an unrestricted autopsy was performed. Only the pertinent findings will be described.

The heart weighed 520 g with approximately 30-50 mL of pericardial fluid. The right dominant coronary arterial systems showed marked atheromas with calcifications. The left ventricular wall thickness was 1.7 cm (normal 1.0-1.5 cm). Sections of the left coronary arterial system showed significant intimal atheromas with intimal proliferation and calcifications with reduction of the luminal diameter of up to 95% in some regions (Fig. 1a). The right coronary artery also showed significant intimal atheromas and calcification with reduction of the interior diameter of up to 50%. Microscopically, portions of the anterior free wall of the left ventricle showed an inflammatory infiltrate consisting predominately of neutrophils with necrosis of the myocardium (Fig. 1b). Additional sections of the anterior wall distant from the inflammatory infiltrate showed a significant patchy, fibrous scarring of the myocardium. Ultrastructural analysis showed the heart muscle separated by thin strands of fibrosis (Fig. 2). The myocytes showed focal loss of filaments and increased number of mitochondria containing tubulovesicular cristae and dense aggregates. Numerous lamellar inclusions were found in myocytes and fibroblasts. Inclusions were only rarely seen in endothelial cells (Fig. 2d).

The intimal surface of the aorta showed marked atherosclerotic ulcerative plaques. The orifices of major arteries were widely patent.

The lungs were heavy and consolidated with bloodtinged pulmonary edema. Pulmonary hemorrhage was identified in the peripheral hilar region of the right and left lung. Microscopic examination showed significant congestion, with hemorrhage into the bronchioles and spilling into the alveolar spaces with distal necrosis/infarction of the parenchyma. Other sections from the consolidated lung showed areas of frothy, eosinophilic cell-free fluid filling the alveolar spaces and scattered, admixed hemosiderinladen macrophages.

The right kidney weighed 140 g and the left one weighed 184 g. The pelvis and calices of both kidneys were markedly dilated with thinning of the renal cortical parenchyma. Microscopically, glomeruli were either normal with occasional mesangial widening or sclerosis (Fig. 3). At the ultrastructural level, numerous lamellar inclusions were present in the glomerular epithelium and tubular epithelial cells. Only an occasional inclusion was seen in endothelial cells of glomerular capillaries (Fig. 4). Glycolipid deposits were not seen in peritubular capillary endothelial cells and only rarely seen in mesangial cells (not shown).

The fixed brain weighed 1,520 g. The left cerebral hemisphere showed a cystic cavity area in the frontoparietal lobes corresponding to an old infarct in the left MCA territory. Microscopically, this area was characterized by abnormal brain parenchyma with abundant reactive astrocytes, few foamy macrophages containing hemosiderin, and calcification. The basilar artery was atherosclerotic. Luxol fast blue (LFB)-stained deposits were found in blood vessels, mostly in the smooth muscle cells of the media and adventitia and little or none in endothelial cells (Fig. 5). Similar results were obtained by electron microscopy (Fig. 6). Neuronal storage was also observed in various areas including the hypothalamus (Fig. 7a), brainstem, and spinal cord. Sections of the paraspinal ganglia showed prominent neuronal distention with accumulating storage deposits (Fig. 7b).

Discussion

We describe herein the pathological abnormalities of a 47-year-old man with Fabry disease who died of acute myocardial infarction associated with nonhypertensive atherosclerotic coronary artery disease. Other significant findings at autopsy were the characteristic Fabry cardiomyopathy with increased cardiac mass by both weight and arterial wall thickness, as well as microscopic fibrosis and marked cellular storage. Typical primary and secondary findings of Fabry disease were also found in the kidney, the brain, and the paraspinal sensory ganglia.

A number of autopsy and pathological reports of patients with Fabry disease have been published [6,7,10,20,21]. The pathological findings in this patient showed an almost complete absence of glycolipid deposits in vascular endothelial cells attributable to the agalsidase beta infusions. Similar findings of predominant clearing of capillary endothelial cells were described in kidney biopsy material in the pivotal trial of agalsidase beta [21]. The presence of marked storage in other cells, including smooth muscle cells and pericytes of the vascular system, as well as in nonvascular cells, after more than 2 years of enzyme infusions suggests that the infused enzyme has limited access to nonvascular endothelial cells. If so, this may constitute a significant limitation of this therapeutic approach. We cannot however exclude the fact that greater storage clearance is achieved in other patients. Efforts to modify recombinant α -galactosidase A or the development of other therapeutic strategies may achieve more complete clearing of storage material. The partial clearance of glycolipid deposits from the vascular tree may explain in part the clinical impression that the incidence of strokes is not reduced in the first few years after α -galactosidase A infusions.

The involvement of the coronary arteries in Fabry disease has been previously recognized [1, 7,9,12], although minimized by others [19]. In addition, the possibility of accelerated atherosclerosis in Fabry disease has been previously mentioned [11,13,15]. Reported autopsies have shown that these coronary lesions are similar to typical atherosclerosis except that they are concentric with a white discoloration rather than the eccentric yellowish characteristics of atherosclerotic plaques [1].

The severe atherosclerotic disease seen in this patient that culminated in a fatal coronary event could be coincidental to the Fabry disease process. Nevertheless, we increasingly recognize fixed coronary artery disease among our Fabry disease patient population. For example, five men with Fabry disease from a group of 38 patients who participated in enzyme replacement trials (age range 19-49, not including this patient) were found to have symptomatic fixed coronary artery disease requiring coronary bypass or angioplasty (three patients). In addition, we found that random (not during acute events) blood levels of myeloperoxidase (MPO) in patients with Fabry disease are significantly higher than controls [15]. MPO has been associated with the atherosclerosis process, endothelial dysfunction, and acute coronary events, and it was recently shown to modify apo A-1 HDL cholesterol to make it more likely to accumulate in macrophages [16]. Evidence for excess production of reactive oxygen species, such as superoxide in Fabry disease, has also accumulated [14,15]. Recently, another group has shown that aortic atherosclerosis in mice deficient in both apo E and α -galactosidase A is markedly accelerated compared with either of those mouse models alone [3]. Together, this clinical and experimental evidence suggests that the process of atherosclerosis may be accelerated in Fabry disease. It is likely therefore that other factors in combination with α -galactosidase A deficiency render certain patients with Fabry disease susceptible to atherosclerosis. Indeed, we have recently shown that polymorphisms of genes known to be risk factors for stroke modify the likelihood of developing cerebral ischemic lesions in Fabry disease [2]. Therefore, a number of genetic factors may modulate the interaction between the α -D-galactosyl glycolipid

accumulation and vascular plaque formation. Additional investigation is necessary to confirm and further characterize this process.

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Fig. 1. Cardiovascular findings. **a** Section of the left coronary artery showing a marked intimal proliferation and stenosis of the lumen. Hematoxylin and eosin (H & E), $\times 20$. **b** Representative section of the anterior portion of the left ventricle showing coagulative necrosis of the muscle with wavy fibers and a dense polymorphonuclear leukocytic infiltrate. H & E, $\times 20$

Fig. 2.

Ultrastructure of the heart. **a** Large aggregates of concentric lamellated cytoplasmic inclusions displace cardiac myofibrils to the periphery of the cell. Interstitial fibrosis and increased numbers of mitochondria are secondary features of the cardiomyopathy. **b** Higher magnification of the lamellar inclusions and the accumulated mitochondria. The latter exhibit tubulovesicular cristae and cell size variation. **c** The myelinoid inclusions sometimes consisted of parallel stacked lamellae with a zebra-like pattern. **d** Inclusions were only rarely present in endothelial cells of adjacent interstitial capillaries (arrows)

Fig. 3.
Representative section of the renal cortex showing a glomerulus with focal mesangial sclerosis.
H & E, $\times 40$

Fig. 4. Ultrastructure of the kidney. **a** Abundant lamellar inclusions in the glomerular epithelial cells (podocytes) and tubular epithelial cells (not shown). **b** On the other hand, only an occasional inclusion was identified in the glomerular capillary endothelial cells (*arrow*)

Fig. 5.
Intracerebral blood vessels with Luxol fast blue (LFB)-stained deposits within vacuolated smooth muscle/adventitial cells. LFB-period acid Schiff (PAS), $\times 100$ oil

Fig. 6.

Ultrastructure of the brain. The smooth muscle cells in the wall of a cerebral vessel shown in a semithin section in **a** (toluidine blue/fuchsin stain) are loaded with lamellar inclusions exhibiting concentric and zebra-like patterns at the ultrastructural level (**b**). Another semithin section (**c**) shows a capillary identified within the cerebral tissue, which exhibits many Fabry-type inclusions in pericytes (*arrows*) but lacks endothelial cell inclusions (**d**)

Fig. 7.
a Storage in neurons. Hypothalamic nuclei showing neuronal storage. H & E, $\times 40$. **b** Dorsal root ganglia showing swollen storage neurons. H & E, $\times 20$