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Original contribution

Increased oxidative stress contributes to cardiomyocyte dysfunction and death in patients with Fabry disease cardiomyopathy☆,☆☆

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Summary Cardiac dysfunction of Fabry disease (FD) has been associated with myofilament damage and cell death as result of α-galactosidase A deficiency and globotriaosylceramide accumulation. We sought to evaluate the role of oxidative stress in FD cardiomyocyte dysfunction. Myocardial tissue from 18 patients with FD was investigated for the expression of inducible nitric oxide synthase (iNOS) and nitrotyrosine by immunohistochemistry. Western blot analysis for nitrotyrosine was also performed. Oxidative damage to DNA was investigated by immunostaining for 8-hydroxydeoxyguanosine (8-OHdG), whereas apoptosis was evaluated by in situ ligation with hairpin probes. iNOS and nitrotyrosine expression was increased in FD hearts compared with hypertrophic cardiomyopathy and normal controls. Remarkably, immunostaining was homogeneously expressed in FD male cardiomyocytes, whereas it was only detected in the affected cardiomyocytes of FD females. Western blot analysis confirmed an increase in FD cardiomyocyte protein nitration compared with controls. 8-OHdG was expressed in 25% of cardiomyocyte nuclei from FD patients, whereas it was absent in controls. The intensity of immunostaining for iNOS/nitrotyrosine correlated with 8-OHdG expression in cardiomyocyte nuclei. Apoptosis of FD cardiomyocytes was 187-fold higher than in controls, and apoptotic nuclei were positive for 8-OHdG. Cardiac dysfunction of FD reflects increased myocardial nitric oxide production with oxidative damage of cardiomyocyte myofilaments and DNA, causing cell dysfunction and death.

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1. Introduction

Fabry disease (FD) is an X-linked lysosomal storage disorder caused by the deficiency of the enzyme α-galactosidase A. It results in progressive intracellular deposition of globotriaosylceramide (GB3) and related neutral glycosphingolipids in multiple organ systems, including skin, kidneys, vascular endothelium, ganglion cells of the peripheral nervous system, and heart [1]. Cardiac involvement is common both in homozygous males and in heterozygous females and contributes substantially to disease-related morbidity and mortality [2,3]. Indeed, the heart can be the only organ involved in the so-called cardiac Fabry variant [4].

Pathologically, GB3 accumulates in all cardiac cell types, including microvascular endothelial and smooth muscle cells, fibroblasts, and cardiomyocytes. Cell GB3 accumulation leads to myocardial ischemia, valvular abnormalities, conduction tissue disease, and myocardial hypertrophy. In particular left ventricular hypertrophy mimics the morphologic and clinical picture of hypertrophic cardiomyopathy [5,6], with early diastolic dysfunction and preserved ejection fraction until the end stage of the disease [7].

Functional and structural alterations of myofilaments and increased cardiomyocyte death seem to significantly contribute to the cardiac dysfunction observed in FD cardiomyopathy [8,9]. However, the mechanism by which intracellular accumulation of GB3 and its metabolites lead to cardiac dysfunction is still unclear [10,11].

Several studies suggest that oxidative stress is implicated in the pathophysiology of FD, particularly for the cardiovascular involvement of the disease. It has been demonstrated that in cultured vascular Fabry endothelial cells, GB3 accumulation leads to increased production of reactive oxygen species (ROS) in a dose-dependent manner [12]. Furthermore, GB3 accumulation induces the expression of intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and E-selectin, whereas reduction of endogenous GB3 leads to decreased expression of adhesion molecules [12]. In human macrovascular and microvascular cardiac endothelial cells, GB3 loading causes deregulation of several key endothelial pathways such as endothelial nitric oxide (NO) synthase, inducible NO synthase (iNOS), cyclooxygenase-1, and cyclooxygenase-2, whereas α-galactosidase A silencing shows no effects [13].

Plasma from FD patients is characterized by increased ROS generation [12], oxidative damage to biomolecules, and reduced antioxidant defenses, assessed by reduced glutathione, glutathione peroxidase activity, and increased superoxide dismutase/catalase ratio in erythrocytes [14]. Moreover, α-galactosidase A deficiency causes increased nitrotyrosine expression in experimental models [15] and in skin biopsies and brain tissue from FD patients [16]. More recently, Shu et al [17] demonstrate that α-galactosidase A knockdown by RNA interference in a human endothelial cell line results in GB3 accumulation, reduced endothelial NO synthase activity, and dramatically enhanced nitrotyrosine production. They also show that elevated levels of nitrotyrosine are found in the plasma and aortic tissue of the α-galactosidase A knockout mouse, as well as in the plasma of untreated men with FD, suggesting the possibility that nitrotyrosine represents a useful biomarker for vasculopathy in FD.

In accordance with the data obtained on FD vascular damage, we hypothesized that increased oxidative stress of cardiomyocytes' myofilaments and DNA might contribute to cardiac dysfunction in FD cardiomyopathy.

2. Materials and methods

2.1. Patient population

Study population included 18 patients with FD, 7 women and 11 men, with a mean age of 49.8 ± 10.4 years. In all patients, the diagnosis of FD was based on the identification of α-galactosidase A mutations and, in males, on the detection of low α-galactosidase A activity in peripheral leukocytes [18].

2.2. Cardiac studies

Extensive clinical examination, including the assessment of FD systemic manifestations, and noninvasive (resting electrocardiogram, echocardiography with tissue Doppler analysis, cardiac magnetic resonance) and invasive cardiac studies were performed in all patients [8]. Invasive cardiac examinations were performed after patient written informed consent and approval by the ethics committee of our institution and included cardiac catheterization, selective coronary angiography, left ventricular angiography, and biventricular or left ventricular endomyocardial biopsy. No patient was on enzyme replacement therapy at the time of endomyocardial biopsy.

2.3. Endomyocardial biopsy

Endomyocardial biopsies (4-5 each ventricular chamber) were performed in the septal-apical region of left or both ventricles. Myocardial samples were processed for routine histologic and histochemical analysis and for transmission electron microscopy [18]. One to 2 endomyocardial biopsy samples were snap-frozen in liquid nitrogen and used for protein analysis.

Surgical left ventricular biopsies from 18 age- and sex-matched subjects with isolated mitral stenosis and normal cardiac function were used as controls. These endomyocardial fragments, although not obtained from healthy individuals, were derived from a not overloaded chamber and were considered the nearest samples to a normal endomyocardial tissue [19]. In addition, left ventricular endomyocardial biopsy from 18 sex- and age-matched patients with hypertrophic