Increased Reactive Oxygen Species and Anti-Oxidative Response in Mitochondrial Cardiomyopathy

Kazunobu Ishikawa, MD; Satoshi Kimura, MD; Atsushi Kobayashi, MD; Takamasas Sato, MD; Hayato Matsumoto, MD; Yuichi Ujiie, MD; Kazuhiko Nakazato, MD; Minoru Mitsugi, MD; Yukio Maruyama, MD

A 60-year-old woman was admitted for treatment of congestive heart failure. She had been diagnosed with diabetes mellitus when she was 23 years old, and she began to go deaf when she was 34 years old. She showed symptoms of heart failure at age 51 and was diagnosed with hypertrophic cardiomyopathy. Echocardiography showed progressive diffuse hypokinetic motion of the left ventricle and the left ventricular hypertrophy had gradually regressed. A mitochondrial transition mutation, A3243G, was detected in her peripheral leukocytes (9%) and in those of her 27-year-old son, who also has diabetes and deafness. Electron microscopy of an endomyocardial biopsy specimen showed proliferation and swelling of the mitochondria, and significant generation of reactive oxygen species (ROS), as well as marked induction of heme oxygenase-1, which is an adaptive enzyme to oxidative damage, were also observed in the myocardial tissue. These observations were more prominent than in other patients with heart failure of different etiology, which suggests that the increased ROS generation and anti-oxidative response were involved in the development of the mitochondrial cardiomyopathy. (Circ J 2005; 69: 617–620)

Key Words: Anti-oxidant; Cardiomyopathy; Heme oxygenase; Mitochondria; Reactive oxygen species

Mutations in mitochondrial DNA (mtDNA), which results in impaired energy production of adenosine triphosphate in the mitochondria of eukaryotic cells, causes mitochondrial respiratory-chain diseases. An A → G transition at position 3243 in the mitochondrial tRNA^Leu(UUR) gene has been identified in patients with cardiomyopathy accompanied by diabetes mellitus and hearing impairment, as well as in the MELAS syndrome (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes). A failure in the electron transport system in the mitochondria is believed to generate intracellular reactive oxygen species (ROS), that provoke the systemic tissue injuries. We demonstrate increased ROS production in the endomyocardial tissues of a patient with mtDNA 3243 mutation, accompanied by significant induction of heme oxygenase-1 (HO-1), an anti-oxidative enzyme, which was probably an adaptive response in the diseased myocardium.

Case Report

A 60-year-old woman was admitted in August 2003 because of exacerbated dyspnea. She had been diagnosed with diabetes mellitus when she was 23 years old and treated with insulin for more than 25 years. Her hearing impairment started when she was 34 years old. She started having shortness of breath at age 51 and was diagnosed with hypertrophic cardiomyopathy. She was small in stature (height: 143 cm; body weight: 28 kg; body mass index: 13.7 kg/m²).

On admission, her blood pressure was 92/60 mmHg, there was an S₃ gallop and marked pulmonary congestion on a chest X-ray. A 12-lead electrocardiogram showed sinus tachycardia, left atrial overload and an intraventricular conduction disturbance (Fig 1, Right panel). Her blood sugar was 198 mg/dl, hemoglobin A1c 7.1%, and brain-type natriuretic peptide 372 (>18.8) pg/ml. An echocardiogram showed diffuse hypokinetic motion of the left ventricle with mild hypertrophy (Fig 2, Right panel), although a previous echocardiogram from 1999 showed that she had had significant left ventricular hypertrophy (Fig 2, Left panel). She was treated with diuretics, digitalis and angiotensin-converting enzyme inhibitor followed by additional treatment with angiotensin type-1 receptor antagonist and \( \beta \)-blocker. Nuclear ventriculography using quantitative gated myocardial single photon emission computed tomography with \(^{99m}\)Tc-tetrofosmin revealed non-dilated systolic dysfunction (end-diastolic volume: 79 ml; end-systolic volume: 56 ml; left ventricular ejection fraction: 29%).

Because of the combination of cardiomyopathy, diabetes and deafness, we screened for mitochondrial disease. Brain computed tomography did not reveal any abnormalities, but an mtDNA transition mutation, A3243G, was confirmed in her peripheral leukocytes (9%) and in her 27-year-old son, who also has diabetes and deafness, supporting maternal inheritance (Fig 3).

After stabilization of her symptoms of heart failure, cardiac function and degeneration of myocardial tissues were assessed by cardiac catheterization. Left ventriculography also revealed the diffuse hypokinetic motion of the left ventricle (ejection fraction: 29%). Coronary angiography did not reveal any significant stenosis. Endomyocardial biopsy specimens from the left ventricle showed marked degenera-

tion of cardiomyocytes with hypertrophic changes and interstitial fibrosis (not shown). Residual cardiomyocytes had hypertrophic changes (mean transverse diameter: 26.8±7.9 μm). Electron microscopy showed proliferation and swelling of the mitochondria in the cardiomyocytes (not shown) and dihydroethidium staining revealed significant generation of ROS in the endomyocardial cells (Fig 4). In addition, immunohistochemical staining showed marked induction of HO-1 (7), which suggests an adaptive response to oxidative damage (Fig 5). To confirm that these increased ROS generation and HO-1 expression are specific for mitochondrial cardiomyopathy, we examined the failing myocardium of the patients due to other etiology such as idiopathic dilated cardiomyopathy (Figs 4B, 5C, 5D) and idiopathic hypertrophic cardiomyopathy (Figs 4C, 5E, 5F). Although increased ROS generation and HO-1 expression were also observed in the endomyocardium of the patients with heart failure to some extent, the degree of ROS generation and HO-1 expression were much prominent in that of the patient with mitochondrial A3243 mutation.

Fig 1. 12-lead electrocardiogram shows left atrial overload and progression of the intraventricular conduction disturbance for 4 years. High voltages in the precordial leads (V1–3) in April 1999 are interpreted as hypertrophic changes of the interventricular septum, which was much less in August 2003. 1 mV = 10 mm.

Fig 2. Serial echocardiograms. In August 2003 there is diffuse hypokinetic motion of the left ventricle with mild hypertrophy, but the patient, who was initially diagnosed as hypertrophic cardiomyopathy, had significant left ventricular hypertrophy in April 1999. IVS, interventricular septum; LVDd, left ventricular diastolic dimension; LVDs, left ventricular systolic dimension; LV PW, left ventricular posterior wall; LVEF, left ventricular ejection fraction; FS, fractional shortening.
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Fig. 3. Family tree of the patient. A mitochondrial DNA transition mutation, A3243G, was confirmed in the peripheral leukocytes in the patient (closed circle) and her 27-year-old son (closed box), suggesting maternal inheritance. The mother of the patient died from heart failure at 71 years old (precise condition unknown).

Fig. 4. Reactive oxygen species (ROS) generation is shown by dihydroethidium staining of the endomyocardial biopsy specimens from the patients with mitochondrial A3243G cardiomyopathy (A), idiopathic dilated cardiomyopathy (B) and idiopathic hypertrophic cardiomyopathy (C). Fresh cryostat sections (30 μm) placed on glass slides were briefly washed, incubated with phosphate-buffered saline (pH 7.4) containing 10 μM dihydroethidium (Sigma-Aldrich, St Louis, MO, USA) and coverslipped. The slides were placed in a light-protected humidified chamber at 37°C for 30 min and ROS generation was evaluated by the formation of ethidium bromide (EtBr). EtBr is excited at 488 nm with an emission spectrum of 610 nm. Images were obtained with a confocal fluorescent microscopy under identical laser settings. Fluorescence was detected with a 585 nm long-pass filter (Magnification (A–C), ×200).

Fig. 5. Expression of heme oxygenase-1 (HO-1), an enzyme responsive to oxidative damage, in endomyocardial biopsy specimens from the patients with mitochondrial A3243G cardiomyopathy (A, B), idiopathic dilated cardiomyopathy (C, D) and idiopathic hypertrophic cardiomyopathy (E, F). HO-1 expression was examined by immunohistochemistry (A, C, E). Control stainings were performed using nonimmune sera instead of primary antibody (B, D, F). An avidin-biotinylated peroxidase system was used for immunohistochemical analyses (Magnification (A–D), ×400).
Cardiomyopathy caused by mtDNA 3243 mutation (A3243G) is usually preceded with diabetes and a hearing impairment that gradually develop between the 20s and 30s. Patients exhibit the variety of symptoms, such as MELAS, diabetes, hearing impairment and cardiomyopathy, because the distribution and percentage of the mutant mitochondrial DNA differs in each organ. Most of these disorders are maternally inherited.

The cardiac involvement is often characterized by morphological changes that are similar to hypertrophic cardiomyopathy, in which abnormal mitochondrial accumulation has been reported. In some cases, there is a fatal conduction disturbance. A recent report has shown that nearly 1% of the patients with diabetes has the mtDNA A3243G mutation, and as more than 90% of patients with mitochondrial cardiomyopathy have a hearing impairment, diabetic patients with a hearing impairment should be examined for the mtDNA abnormality and cardiac involvement. Diagnosis is confirmed by genetic analysis and electron microscopic analysis of myocardial biopsy specimens. Although administration of coenzyme Q10 has been used to improve impaired metabolism in mitochondria, the effectiveness of this pharmacotherapy has not been proven. Accordingly, treatment consists of supportive therapies for the diabetes and heart failure.

The significant ROS generation detected by dihydroethidium staining may be interpreted as increased oxidative stress in the myocardium and in the present case was accompanied by marked expression of HO-1. Although administration of coenzyme Q10 has been used to improve impaired metabolism in mitochondria, the effectiveness of this pharmacotherapy has not been proven. Accordingly, treatment consists of supportive therapies for the diabetes and heart failure.

The significant ROS generation detected by dihydroethidium staining may be interpreted as increased oxidative stress in the myocardium and in the present case was accompanied by marked expression of HO-1. Although these observations are not specific to patients with mitochondrial cardiomyopathy, the ROS generation in the endomyocardial biopsy specimen of the present case was significantly increased in comparison with cardiomyopathies of other etiologies, which suggests that the myocardial degeneration is mediated by the ROS produced by the mitochondria in which electron transport is severely impaired because of the mitochondrial DNA A3243G mutation.

Acknowledgements
Pathological work of this case report was supported by Grant-in-aid (14570682 and 16590703) from the Ministry of Education, Science, and Culture of Japan.

References