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Evaluation of respiratory chain failure in mitochondrial cardiomyopathy by assessments of ^{99m}Tc-MIBI washout and ^{123}I-BMIPP/^{99m}Tc-MIBI mismatch

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Abstract

Cardiomyopathy is one of the main features that determines prognosis in patients with mitochondrial encephalomyopathy. We investigated respiratory chain failure using ^{99m}Tc-MIBI- and ^{123}I-BMIPP-SPECT *in vivo* in five patients with mitochondrial cardiomyopathty. With the lowering of cardiac function, the ^{99m}Tc-MIBI-washout rate (WOR) increased, and the ^{99m}Tc-MIBI-uptake decreased, conversely. In patients who showed severe cardiac involvement, ^{99m}Tc-MIBI-uptake was markedly reduced, and by contrast, ^{123}I-BMIPP-uptake increased (^{123}I-BMIPP/^{99m}Tc-MIBI mismatch). There were significant correlations between the WOR on ^{99m}Tc-MIBI-SPECT and interventricular septal thickness (IVST) on echocardiography and between WOR and left ventricular ejection fraction (LVEF) on ^{99m}Tc-MIBI-SPECT. The increased WOR and decreased uptake of ^{99m}Tc-MIBI were reflected by the lowered mitochondrial membrane potential created by mitochondrial respiratory chain whereas ^{123}I-BMIPP/^{99m}Tc-MIBI mismatch may be created by the enhanced triglyceride-pool. These nuclear medicine techniques are the potential tools to evaluate the energy state in mitochondrial cardiomyopathy.

Keywords: Mitochondrial cardiomyopathy; Mitochondrial DNA; A3243G mutation; ^{99m}Tc-MIBI washout rate; ^{123}I-BMIPP/^{99m}Tc-MIBI mismatch

1. Introduction

The heart relies exclusively on the mitochondrial respiratory chain for energy for construction and dilatation. Cardiomyopathy has been observed as a common feature in patients with mitochondrial diseases caused by mitochondrial DNA (mtDNA) mutations, and restricts the prognosis as well as encephalopathy (Goto et al., 1990; Vilarinho et al., 1997; Silvestri et al., 1997; Shiotani et al., 1998).

An A-to-G transition mutation at nucleotide position 3243 (A3243G) was originally discovered in patients with mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS) syndrome (Goto et al., 1990), and is the most common cause of mitochondrial cardiomyopathy (Vilarinho et al., 1997; Silvestri et al., 1997; Shiotani et al., 1998). Cardiomyopathy can be either heart-specific or in association with a spectrum of other MELAS symptoms (Vilarinho et al., 1997; Silvestri et al., 1997; Shiotani et al., 1998; Anan et al., 1995). In mitochondrial diseases, the mutant mtDNA is usually mixed with the wild-type mtDNA in a cell or among tissues, i.e. heteroplasmy (Chomyn et al., 1992; Yoneda et al., 1994; Holt et al., 1988; Tanno et al., 1995). The accumulation of mutant mtDNA causes respiratory chain failure when the proportion of the mutant mtDNA

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exceeded the threshold for functional complementation by wild-type mtDNA in a cell (Chomyn et al., 1992; Yoneda et al., 1994). There are two possible pathogeneses on mitochondrial cardiomyopathy; one is metabolic failure in the myocardium (cytopathy), another is ischemia caused by arterial mitochondrial dysfunction (angiopathy) (Sato et al., 1994; Yoneda et al., 1989; Sakuta and Nonaka, 1989).

Although evaluation of respiratory chain failure is clinically important in patients with mitochondrial cardiomyopathy, there are no useful tools available for this evaluation *in vivo*. Recent studies demonstrated that technetium 99m methoxyisobutyl isonitrile (^{99m}Tc-MIBI) was incorporated and retained in the mitochondria of myocardial cells, depending on the mitochondrial membrane potential ($m\Delta\psi$) created in the respiratory chain (Okada et al., 1988; Konno and Kako, 1991; Piwnica-Worms et al., 1990; Chiu et al., 1990; Carvalho et al., 1992; Crane et al., 1993). An iodine 123-labeled 15-4-iodophenyl-3-(*R*,*S*)-methyl-pentadecanoic (^{123}I-BMIPP), a modified synthetic fatty acid, is known to be a tracer associated with suppressed fatty acid metabolism with enhanced glucose utilization (Fujibayashi et al., 1988; DeGrado et al., 1989; Fujibayashi et al., 1990; Knapp et al., 1990; Takeishi et al., 1997).

In this study, we evaluated changes in the energy state due to respiratory chain failure in cardiomyopathic patients due to the MELAS A3243G mutation using ^{99m}Tc-MIBI- and ^{123}I-BMIPP-single photon emission tomography (SPECT).

2. Methods

2.1. Study subjects

We studied five patients who presented with cardiomyopathy and carried the MELAS A3243G mutation. The clinical and cardiac features of these patients were summarized in Table 1. Cardiac function was evaluated by New York Heart Association (NYHA) class, chest X-ray (cardiac/thoracic ratio; CTR), ECG, echocardiography and serum B-type natriuretic peptide (BNP). The human ethics committee of the University of Fukui approved this study protocol, and written informed consent was obtained from each patient.

2.2. Echocardiography

Two-dimensional and M-mode Doppler echocardiography was performed in all patients using a SONOS 5500 ultrasound system with a 2.5 MHz transducer (Phillips Medical Systems, Andover, MA). Left ventricular end-diastolic diameter (LVDd), left ventricular end-systolic diameter (LVSd), interventricular septal thickness (IVST) and posterior wall thickness (PWT) were measured on M-mode images obtained from the parasternal long-axis view and the percent fractional shortening (%FS) was calculated using the formula: $FS = (LVDd - LVSd)/LVDd \times 100$. Systolic impairment was defined as $FS < 30\%$.

2.3. ^{99m}Tc-MIBI-SPECT and ^{123}I-BMIPP-SPECT imaging

All images were acquired with a dual-head rotating gamma camera (e.cam. signature, Siemens-Asahi, Tokyo, Japan). For collimation, a low energy high resolution collimator was used for ^{99m}Tc-MIBI imaging and low and medium energy collimator for ^{123}I -BMIPP imaging. A dose of 600 MBq of ^{99m}Tc-MIBI (Daiichi Radioisotope Laboratories Ltd, Tokyo, Japan) was administered intravenously under resting and overnight fasting conditions. The planar images were obtained 60 min (early image) and 240 min (delayed image) postinjection. Mean mediastinal counts (M) and mean heart counts (H) were obtained from each of early and delayed planar images. The washout rate (WOR) was calculated as follows: WOR (%) = [(H early − M early) − (H delayed − M delayed)]/(H early − M early) × 100 (normal range is less than 15%). ECG-gated SPECT was acquired just after the acquisition of early planar image. The gamma camera rotated, collecting 72 projections (22 seconds per projection) over 360 degree. The projection data were reconstructed into 64×64 matrix images using the filtered-back projection method with a Butterworth filter (order 10, cutoff 0.6 cycle/pixel) and a ramp filter. For gating, 12 frames per cardiac cycle with re-fixed RR intervals and 15% window (140 keV of photopeak) were used. Left ventricular ejection fraction (LVEF) was calculated using a quantitative gated SPECT (QGS) program.

Each patient received an injection of 111 MBq of ^{123}I-BMIPP (Nihon Medi-Physics Co., Ltd, Hyogo, Japan). Thirty minutes after the injection, data acquisition was performed. For ^{123}I-BMIPP imaging, a low and medium energy collimator was used, which is suitable for the imaging of iodine-123 labeled tracers. Energy window was 15% and the photopeak was 159 keV. The number of projection was 60, and the projection data were reconstructed into 128×128 matrix images using the filtered-back projection method with a Butterworth filter (order 8, cutoff 0.3 cycle/pixel) and a ramp filter.

After the image reconstruction, each transaxial image was re-oriented, and short-axis, vertical-long axis, and horizontal-long axis images were generated. Using these images, visual analysis of ^{99m}Tc-MIBI and ^{123}I-BMIPP uptakes was performed separately by two independent observers who were unaware of the patient data.

2.4. Statistical analysis

Simple correlations between WOR on ^{99m}Tc-MIBI-SPECT and IVST on echocardiography and between WOR and LVEF on ^{99m}Tc-MIBI-SPECT were determined. A value of $P < 0.05$ was considered statistically significant.