Clinical and Electron Microscopic Findings in Two Patients with Mitochondrial Myopathy Associated with Episodic Hyper-creatine Kinase-emia

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Abstract

Mitochondrial myopathy with episodic hyper-creatine kinase (CK)-emia (MIMECK) is a new disease entity characterized by episodic or persistent muscle weakness and elevated CK levels. We herein report two cases of MIMECK with the findings of histopathological studies. Histopathological examinations revealed strongly succinate dehydrogenase-reactive vessels. Electron microscopy showed abnormal mitochondria in the vessels and proliferating and vacuolated mitochondria under the sarcolemma. Both patients exhibited recurrent severe myalgia, weakness and increased CK levels. L-arginine treatment significantly ameliorated their muscle symptoms. These findings indicate that mitochondrial angiopathy plays an important role in the pathophysiology of MIMECK. L-arginine may be a potential therapeutic agent for this disorder.

Key words: mitochondrial myopathy, elevated creatine kinase, arginine


Introduction

Mitochondrial diseases have a varied clinical presentation and can occur at almost any stage of life (1). Diagnosing primary mitochondrial disorders can be challenging, and biochemical and/or molecular evaluations are often necessary to confirm a specific diagnosis (2). Histological studies of muscle biopsies are a main tool used to identify mitochondrial diseases (3). A mosaic or global pattern of cytochrome c oxidase (CCO)-deficient fibers or the presence of ragged red fibers (RRFs) are key histochemical features of mitochondrial myopathies. In cases of mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS), strongly succinate dehydrogenase (SDH)-reactive blood vessels (SSVs) are noted, caused by the abnormal accumulation of mitochondria in the blood vessels (4). We previously reported a novel mitochondrial disease subtype, mitochondrial myopathy with episodic hyper-creatine kinase (CK)-emia (MIMECK). Individuals with this disease carry 16 mitochondrial DNA (mtDNA) alterations and experience episodic or persistent muscle weakness and elevated serum CK levels triggered by infection, drugs or stressful situations (5). We hypothesized that these genetic alterations work concomitantly to modify the mitochondrial function; however, the precise pathogenic mechanism has not yet been elucidated, and no effective treatment has been reported. Despite significant progress in our understanding of the biochemical and molecular basis of mitochondrial disease, physicians are still limited in their ability to treat such conditions. Tested pharmacological agents include respiratory chain cofactors, antioxidants and drugs that decrease lactic acid accumulation; however, no clear evidence supporting the use of any such interventions has been found (6). Gene therapy is currently being studied, and one important strategy is to decrease the ratio of mutant to wild-type mitochondrial genomes, a method known as “gene shifting” (7). Nevertheless, this technique has not yet been tested clinically to date.

According to the hypothesis that stroke-like episodes in MELAS patients are caused by impaired vasodilatation in intracerebral arteries, L-arginine has a therapeutic effect on...
MIMECK, as it improves the function of smooth muscle and endothelial cells in arteries (4). In this study, we examined muscles and vessels obtained from two MIMECK patients using histochemical methods and electron microscopy and found abnormal mitochondria in the vessels. Since MIMECK is characterized by acute muscle damage, the acute mitochondrial dysfunction resembled stroke-like episodes in MELAS. Because of the abnormalities in the vessels and the similarity in onset of these two diseases, we decided to use L-arginine to treat our two MIMECK patients, which led to significant amelioration of their muscle symptoms.

Case Reports

Patients

Patient 1

Patient 1 was a 32-year-old woman who had experienced transient limb weakness and general fatigue once a year since 25 years of age. Her mother had previously been diagnosed with MIMECK. At 29 years of age, the patient developed myalgia and muscle weakness. Her serum CK level was high, and a muscle biopsy was performed. She was suspected of having mitochondrial disease, which was subsequently confirmed on histopathological studies. However, we detected no causative mtDNA mutations at that time. At 32 years of age, four weeks before the current admission, she presented with limb weakness and myalgia after bathing in the sea. Three days before admission, her symptoms worsened, and she found it difficult to walk unaided. On admission, she complained of severe myalgia and generalized muscle weakness and was nearly bedridden. Her serum CK level was 959 IU/L (normal range, 45-163 IU/L), although her lactate and pyruvic acid levels were normal [lactate, 4.4 mg/dL (normal range, 3.7-16.3 mg/dL); pyruvic acid, 0.4 mg/dL (normal range, 0.3-0.9 mg/dL)]. Acute exacerbation of MIMECK was diagnosed after taking her mother’s history into consideration. L-arginine (0.5 g/kg) was administered intravenously. The next day, her myalgia and weakness were significantly ameliorated, and she could walk unaided. Her serum CK level decreased to 88 IU/L by the seventh hospital day. After discharge, she experienced episodic myalgia, and her serum CK level shifted between 100 and 500 IU/L. At 34 years of age, she suffered from severe myalgia and weakness such that she could not walk. She also complained of dyspnea, and her serum CK level was elevated at 1,339 IU/L. She received intravenous L-arginine treatment, and her condition improved immediately. Eight months later, the muscle symptoms recurred. She received outpatient treatment and maintained a rest period; however, her symptoms drastically worsened, with a serum CK level of 2,888 IU/L. She was hospitalized, and intravenous L-arginine treatment was initiated. Her symptoms abated and the serum CK level decreased within a few days (Fig. 1).

Patient 2

Patient 2 was a 76-year-old man who had begun to experience delusions of persecution and auditory hallucinations at 22 years of age. At 66 years of age, he presented with myalgia and generalized weakness. Her serum CK level was 5,518 IU/L, and he was initially diagnosed with polymyositis at another hospital. When he presented at our hospital, a muscle biopsy was performed. A pathological examination revealed myopathic...
changes and mitochondrial abnormalities. He complained of recurrent episodic weakness and myalgia, and his serum CK level increased to as high as 11,708 IU/L during episodes of decreased immunity, including minor episodes such as a common cold. At 76 years of age, he presented with lower limb weakness and dysphagia 10 days before admission. One week before admission, he developed a fever, cough and sputum production. On admission, he was bedridden because of severe generalized weakness and myalgia. His laboratory findings were as follows: serum CK level, 360 IU/L; C-reactive protein level, 1.27 mg/dL (normal range, <0.3 mg/dL); lactate level, 22.0 mg/dL (normal range, 3.7-16.3 mg/dL); pyruvic acid level, 1.2 mg/dL (normal range, 0.3-0.9 mg/dL); cerebrospinal fluid level, normal; lactate and pyruvic acid levels, normal. A chest radiograph showed right lower lobe consolidation. A diagnosis of acute exacerbation of MIMECK with aspiration pneumonia was made. L-arginine (0.5 g/kg) and an antibiotic were administered intravenously. The patient’s muscle weakness and myalgia were subsequently ameliorated, and his serum CK level decreased to 153 IU/L by the eighth hospital day, following which he could walk with the aid of a wheeled walker.

Pathology studies

Histopathological studies for patient 1 revealed moderate variation in the muscle fiber size with no necrotic fibers. Several RRFs were detected in the mGT-stained sample. CCO-deficient fibers were detected, and highly expressed fibers were observed in the SDH-stained sample (Fig. 2). SSVs were observed and found to be strongly positive on complex I, II, III, IV and V staining (Fig. 3). Electron microscopy revealed an abnormal proliferation of normal-shaped mitochondria and vacuolated mitochondria under the sarcolemma (Fig. 4a). Enlarged mitochondria in vascular endothelial cells were also detected (Fig. 4b).

As we reported previously, histopathological studies for patient 2 revealed several RRFs in the mGT-stained sample and highly expressed fibers in the SDH-stained sample. However, we detected no SSVs in the specimens. Electron microscopy revealed significant proliferation of mitochondria and accumulation under the sarcolemma; in some sections, mitochondria were present at high densities (Fig. 4c).

Genetic studies

A mitochondrial DNA analysis revealed the same 16 alterations that were previously reported in patients 1 and 2: np200, np257, np1442, np4612, np5127, np6332, np7389, a 9bp deletion between np8281 and 8289, np8291, np10403, np11151, np11969, np13105, np16325, np16390 and np16523 (5).
Figure 3. Immunohistochemical results. Samples stained for (a) succinate dehydrogenase (SDH), (b) Complex I, (c) Complex II, (d) Complex III, (e) Complex IV and (f) Complex V. Strongly SDH-reactive vessels (SSVs) are observed and are strongly positive on Complex I-V staining. (a)-(f): bar, 100 μm.

Figure 4. Electron microscopy images. (a), (b) Patient 1. Abnormal proliferation of normal-shaped mitochondria (arrows) and vacuolated mitochondria (arrowheads) under the sarcolemma are observed. Enlarged mitochondria (arrowheads) are also observed in vascular endothelial cells. (c) Patient 2. Significant proliferation of mitochondria and accumulation under the sarcolemma. In some sections, the mitochondria are present at high densities. (a), (c): bar, 1 μm; (b): bar, 500 nm.
Discussion

In this study, we presented evidence of mitochondrial abnormalities in vessels using histopathological and electron microscopy studies in a MIMECK patient (patient 1). Although the underlying mechanisms are not completely understood, MIMECK is characterized by acute muscle damage. The acute mitochondrial dysfunction observed in cases of MELAS and MIMECK appears to be similar. L-arginine is used to treat the stroke-like episodes of MELAS, which are caused by pathological vasodilatation. Because of the abnormalities in vessels and similarity between these two diseases, we decided to treat our patients with L-arginine. In response, their muscle symptoms and elevated serum CK levels decreased quickly and significantly.

We previously reported that the muscle pathology in patients with MIMECK, including that of patient 2, shows RRFs, highly expressed SDH-stained fibers and CCO-deficient fibers (5). In this study, RRFs, highly expressed SDH-stained fibers and CCO-deficient fibers were also detected in patient 1, who was younger than patients evaluated in previous studies (5) and underwent a muscle biopsy at 29 years of age. The ratios of RRFs and CCO-deficient fibers were low. However, these findings were unusual for her age. Electron microscopy revealed abnormal proliferation under the sarcolemma in both patients. These abnormalities confirmed the pathological diagnosis of mitochondrial myopathy. In patient 1, histochemical studies showed SSVs, and electron microscopy demonstrated abnormal mitochondria in the vessels. SSVs are characteristic of MELAS and are caused by the accumulation of abnormal mitochondria in blood vessels. In small arteries or arterioles, this accumulation can result in segmental occlusion, causing stroke-like episodes (4, 8). Mitochondrial abnormalities in perivascular smooth muscle and endothelial cells were detected on muscle pathology in patient 1. The defective mitochondrial function in the vessels might cause acute muscle damage. We found no SSVs in nine MIMECK patients in our previous report. Patient 1 showed rapidly worsening muscle symptoms, and we therefore detected SSVs. The symptoms of patient 2 were quickly ameliorated after L-arginine administration. Our results suggest that mitochondrial angiopathy plays a key role in the pathophysiology of MIMECK.

We hypothesize that similar pathogenic mechanisms underlie the acute phases of MELAS and MIMECK. The pathogenic mechanism of stroke-like episodes in MELAS remains controversial and may be complicated by mitochondrial angiopathy, mitochondrial cytopathy or a combination of both (4). MELAS is presumably caused by impaired vasodilation in intracerebral arteries; therefore, the therapeutic effect of L-arginine may be related to its function as a precursor of nitric oxide, an important mediator of cerebral vasodilatation. L-arginine also decreases ischemic damage during the acute phase of focal brain ischemia by increasing microcirculation in the cerebral blood flow (9). Because arginine is required for creatine synthesis, an improved understanding of the significant role of nitric oxide and creatine in the central nervous, muscular and vascular systems will broaden the scope of indications for arginine treatment (10). Mitochondrial myopathy generally involves chronic progressive muscle weakness and exercise intolerance (11), whereas MIMECK is characterized by atypical clinical presentations of acute or subacute episodic muscle weakness and myalgia. Although the pathogenic mechanism of acute-phase MIMECK is not clear, mitochondrial dysfunction similar to that seen in the central nervous system (CNS) of MELAS patients may also occur in the muscles of MIMECK patients. We therefore treated our patients with L-arginine, which ameliorated their symptoms, suggesting that L-arginine may be useful for the treatment of MIMECK. During her third attack, patient 1 was treated with rest and ordinary transfusions as an outpatient; however, her muscle symptoms rapidly deteriorated until she was nearly bedridden, with a rise in her serum CK level to as high as 3,000 IU/L. Treatment with intravenous L-arginine promptly improved her condition, more rapidly than the anticipated natural course to remission.

Patient 2 experienced progressive generalized weakness over the course of approximately 10 years. In the early stage, his muscle strength was normal during asymptomatic intervals, and his serum CK level increased to 11,708 IU/L during the acute phase. He repeatedly suffered episodic muscle symptoms and CK elevation, gradually developing permanent muscle weakness, atrophy and dysphagia. Acute symptoms can improve without treatment, although progressive permanent muscle weakness over the long term is unavoidable. Therefore, the treatment of muscle symptoms during the acute phase should be accompanied by a warning of progressive increase in muscle symptoms over time. Appropriate guidance is necessary to avoid triggers that exacerbate the disorder. Because the number of attacks was greater in patient 1, her muscle symptoms were more severe. Her muscle strength has been preserved during the interictal phase thus far; however, we should pay close attention to her condition. Some patients with other mitochondrial diseases experience subacute general fatigue of unknown cause and show increased serum CK levels. These symptoms may resemble those of MIMECK.

In conclusion, we herein described a potential treatment for acute-phase mitochondrial myopathy. Our results are limited by the small number of cases included in the current study and the lack of biochemical data. However, we showed that mitochondrial angiopathy plays an important role in the pathophysiology of MIMECK. The use of L-arginine therapy for the muscle symptoms of mitochondrial diseases has not been previously reported to our knowledge. We believe that studies including a larger number of patients will provide more information about the efficacy of L-arginine therapy.

The authors state that they have no Conflict of Interest (COI).
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