[J Tokyo Wom Med Univ
83 (Extra) E98～E102 (2013)]

Original

Mitochondrial Morphology Differences among Cells Comprising the Blood-brain Barrier and Neurons in MELAS; Implications of Opening of Capillary Endothelial Tight Junctions in Neuroprotection

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(Accepted December 26, 2012)

Chronological changes in the morphology of mitochondria in capillary endothelial cells within muscles and cells comprising the blood-brain barrier (BBB) were examined as indicators of mitochondrial dysfunction and adenosine triphosphate (ATP) depletion in a patient with mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS). Electron microscopic study of muscles at biopsy and autopsy and the cerebral frontal cortex at autopsy revealed distinct mitochondrial morphology differences among capillary endothelial cells, cells comprising the BBB and neurons. Biopsied muscle at age 5 years showed scattered disruption of endothelial tight junctions. On the other hand, at age 13 years, capillary endothelial cells in autopsied muscle were inflated with closed tight junctions, and some mitochondrial cristae showed concentric whorling.

Mitochondrial size and number were increased in cerebral capillary endothelial cells, pericytes and astrocytes, while mitochondria in neurons were preserved. At age 13 years, glycogen granules were noted in the intermyofibrillar cytoplasm, cerebral endothelial cells and neurons other than astrocytes. These differences in changes among cells may suggest sequential neuroprotective responses to chronic lactic acidosis and ATP depletion, involving active opening of capillary endothelial tight junctions, in MELAS.

Key Words: lactic acidosis, blood-brain barrier (BBB), tight junctions, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS), neuroprotection

Introduction

Capillary endothelial cells form a permeability barrier between circulating blood and interstitial tissue. Also, the endothelial cells in the brain are components of the blood-brain barrier (BBB) along with pericytes and the perivascular feet of astrocytes, which regulate the access of various substances to neurons. Astrocytes have processes in contact with both capillaries and neurons[1)2)].

We previously described disruption of capillary endothelial tight junctions, or the zona occludens, as the critical pathology of mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS), being the early capillary endothelial cell response to chronic lactic acidosis, in biopsied muscle and autopsied brain tissue from a patient with MELAS[3)].

Herein, we studied tight junctions in capillary endothelial cells and mitochondrial morphology changes as indications of mitochondrial dysfunction and adenosine triphosphate (ATP) depletion[4)5)] of both biopsied and autopsied muscles, and in cells comprising the BBB obtained from the frontal cortex at autopsy. Tight junction disruption might be an early response maintaining brain and muscle homeostasis under conditions of ATP depletion and

sustaining muscular and neuronal survival in chronic lactic acidosis.

Case Report

The clinical, biochemical and brain findings at autopsy of our patient were previously described in detail[3]. This girl had begun to show fatigability at 3 years of age, with symptoms then showing progressive worsening. At 5-years 1-month of age, biopsied muscle revealed low activity of cytochrome *c* oxidase on histochemical staining, and a point mutation of A3243G mitochondrial DNA. Staining for succinate dehydrogenase revealed strong reactivity for this enzyme on blood vessels. At age 13 years and 6 months, she died of multiple organ failure and chronic respiratory failure. Her height had been 104.5 cm (−0.6 SD) and weight 13.07 kg (−2.0 SD) at the age of 5 years and 1 month, and remained stunted at 139 cm (−3.0 SD) and 29.5 kg (−2.4 SD), respectively, at the time of death.

Results

1. Electron microscopic findings of biopsied quadriceps femoris muscle

Muscle biopsy at age 5 years had shown scattered disruption of endothelial tight junctions. Four out of 7 tight junctions in 3 capillaries (57%) were observed to be open. There was no mitochondrial proliferation in endothelial cells, pericytes, intermyofibrillar cytoplasm or subsarcolemmal spaces. Glycogen granule accumulation was seen in subsarcolemmal spaces and in the myofibrillar cytoplasm.

2. Autopsy findings

Autopsy was performed 10 hours after death. The postmortem brain examination revealed diffuse atrophy. Multiple old and recent necrotic foci were observed throughout the cerebrum and were prominent in the bilateral occipital and frontal lobes, along with neuronal loss associated with capillary proliferation, while some neurons were preserved.

3. Electron microscopic findings of the autopsied intercostal muscle

Capillary endothelial cells and pericytes showed irregular inflation and thickening associated with narrowing of the capillary lumen, but no disruptions were detected in 11 tight junctions in 4 capillaries. There was no proliferation of mitochondria in either capillary endothelial cells or pericytes. However, mitochondria were increased in size and number in the subsarcolemmal spaces and myofibrillar cytoplasm. Some mitochondrial cristae were disarranged such that they showed concentric whorling. There were abnormally increased accumulations of glycogen granules and lipid droplets in the intermyofibrillar cytoplasm. Myofibrils were atrophic.

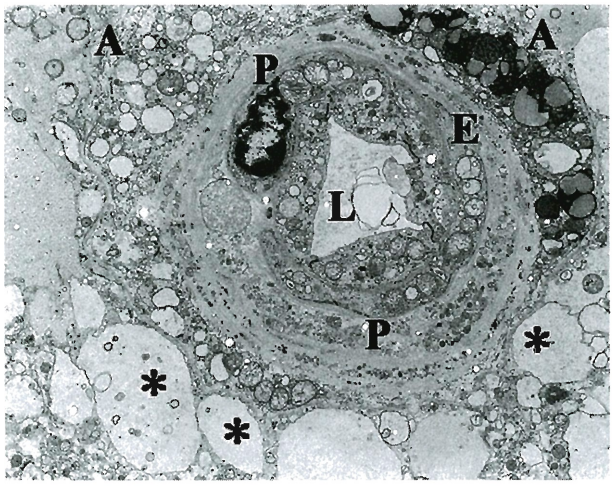

Fig. 1 Electron micrograph of autopsied frontal cortex obtained at age 13 years and 6 months. Capillary endothelial cells (E), pericytes (P) and an astrocytic end-foot (A) are inflated. Distinct narrowing of the lumen (L) is also evident. Some proliferative astrocytes show vacuolation (asterisk). A few lipofuscin granules can be seen in an astrocyte. (×3,500)

4. Electron microscopic findings of the autopsied cerebral frontal lobe

Capillary endothelial cells, pericytes, astrocytes and neurons in the frontal lobe cortex, which was the predominantly affected site with multiple old and recent necrotic foci, were examined. Endothelial tight junction disruption was confirmed in one of 21 tight junctions (5%) in 7 capillaries. Capillary endothelial cells were inflated, mitochondria were increased in size and number; disarrangement of mitochondrial cristae was marked in astrocytic endfeet as well as in endothelial cells and pericytes (Fig. 1). In contrast, neurons were preserved with no alterations of mitochondria (Fig. 2). Similarly, abnormal increases in mitochondrial size and number were also identified mainly in smooth muscles of small arterioles as well as in endothelial cells (not shown). Some of the inflated capillary endothelial

cells and neurons contained glycogen granules. Most astrocytes showed vacuolation (Fig. 1).

The comparison of mitochondrial changes in each of the cell types in muscles and the brain are summarized in Table.

Discussion

This female MELAS patient had a mtDNA A3243G mutation with cytochrome *c* oxidase deficiency. She showed short stature, fatigability, muscle weakness, elevated lactate and pyruvate in both blood and cerebral spinal fluid, stroke-like episodes, psychomotor deterioration, epileptic seizures, blindness, congestive cardiomyopathy and nephrotic syndrome. Short stature is a common and early finding of mitochondrial disease, and is caused by primary hypothalamic growth hormone-releasing hormone deficiency[6].

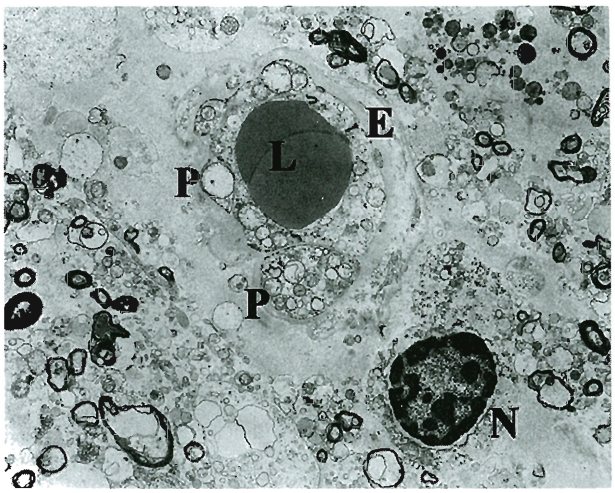

Fig. 2 Electron micrograph of autopsied frontal cortex. Capillary endothelial cells (E) are inflated, the lumen (L) is reduced in size. Pericytes (P) show increased mitochondrial size and number, while a surviving neuron (N) appears normal with glycogen granules. (×3,500)

The BBB is essential to maintaining neuronal viability, and with substantial barrier loss in the hypothalamus[1] there is critical exposure to circulating lactate. Furthermore, decreased intracellular pH affects hypothalamic neuroendocrine cells and suppresses endocrine secretion in MELAS[6]. BBB and capillary endothelial tight junctions may play critical roles in neuronal homeostasis and neuroprotective responses to the chronic lactic acidosis characteristic of MELAS.

Muscle biopsy at age 5 years prior to the distinct onset of stroke-like episodes had shown scattered disruption of endothelial tight junctions, but no mitochondrial abnormalities were found in these endothelial cells. At autopsy, capillary endothelial cells in the intercostal muscles were inflated, with neither

Table Summary of electron microscopic study results

Cell subtype & findings \ Specimen	Muscle Biopsy		Muscle obtained at autopsy		Frontal cortex obtained at autopsy	
Capillary endothelial cells						
opening of tight junctions	+	scattered, 57%	−	0%	+	rarely, 5%
proliferation of cells	−		−		−	
inflated cells	−		+/++		+/++	with glycogen granules
mitochondrial change	−		−		++	increased in size & number
Pericytes						
proliferation of cells	−		−		+	
inflated cells	−		+		+/++	
mitochondrial change	−		−		++	increased in size & number
Astrocytes						
proliferation of cells					+	reactive astrocytosis with/without vacuolation
inflated cells					++	with lipofuscin granules
mitochondrial change					+++	increased in size & number
Neurons						
proliferation of cells					−	
inflated cells					−	with glycogen granules
mitochondrial change					−	

disruption of tight junctions nor mitochondrial abnormalities. Scattered endothelial tight junction disruptions in biopsied muscle, seen at 5 years of age, were no longer detectable in autopsied muscle at age 13 years, suggesting the disruption of tight junctions to possibly be an early neuroprotective response to lactic acidosis. Disruption of endothelial tight junctions was rare in brain capillaries at autopsy (1/21; 5%). Electron microscopic study of the frontal cortex revealed distinct mitochondrial morphology differences in endothelial cells and cells comprising the BBB. Mitochondrial size and number were increased in capillary endothelial cells, pericytes and astrocytes, while mitochondria in neurons were unchanged.

The tight junction disruptions induced by lactic acidosis may represent early and active opening. This active opening of tight junctions might lead to an influx of plasma lactate into the cerebral interstitial space, and the resultant lowering of cerebral interstitial pH would suppress the activities of both cellular enzymes and glycolysis, thereby contributing to neuronal survival associated with low activity. When cerebral metabolism is suppressed in lactic acidosis. lactate transport across the BBB might be enhanced and lactate may partially replace glucose as a substrate for oxidation in astrocytes and neurons[7]. Energy sources for astrocytes include glucose supplied via endothelial cells by specific glucose transporters[2].

Moderate lactic acidosis offers potential neuroprotection through suppression of neuronal activity during ischemia which might reduce neuronal metabolic energy demands and glucose utilization by inhibiting phosphofructokinase[8]. In the early stage of lactic acidosis in MELAS, tight junctions might mediate a neuroprotective action, like hypothermia, against hypoxic-ischemic encephalopathy[9].

Glycogen metabolism usually couples astrocytes and neurons[10]. Glycolysis in astrocytes leads to the production of lactate, which serves as an energy substrate for oxidative metabolism in active neurons mediated by the monocarboxylic acid transporter[10]. However, lactic acidosis in MELAS may suppress glycolysis in astrocytes, and lactic transport from astrocytes to neurons, thereby lowering neuronal activity. Accumulation of glycogen granules was noted in subsarcolemmal spaces and myofibrillar cytoplasm in both biopsied and autopsied muscles, as well as in endothelial cells and neurons in autopsied brain tissue from this patient, indicating ATP demand and glycolysis in muscles and neurons to be reduced.

Thus, in chronic cerebral lactic acidosis, suppressed neuronal metabolism could compensate for ATP depletion in neurons and delay neuronal loss, thereby providing neuroprotection.

Conclusion

The morphological changes in the mitochondria of cells comprising the BBB and neurons were cell-selective in MELAS. The disruption of endothelial tight junctions, as well as these cell-selective changes in mitochondria and surviving neurons with inactivity, may be attributable to neuroprotective responses to chronic cerebral lactic acidosis and ATP depletion.

We obtained no financial support nor do we have any relationships that may pose conflicts of interest.

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MELASにおける血液脳関門構成細胞と神経細胞のミトコンドリア形態の差異；毛細血管内皮細胞の閉鎖結合開放による神経保護の関与

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Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes（MELAS）の1例における，ミトコンドリア機能低下とATP枯渇を表わす指標としてミトコンドリア形態の経時的変化を，筋肉毛細血管内皮細胞と血液脳関門（BBB）構成細胞で検討した．電子顕微鏡所見は，生検筋肉と剖検筋肉の毛細血管内皮細胞と剖検における大脳前頭葉皮質BBB構成細胞および神経細胞間で，ミトコンドリア形態に著しい差異を認めた．5歳時の筋生検は内皮細胞閉鎖結合破綻が散在していた．一方，13歳時の剖検筋肉の毛細血管内皮細胞は，膨化し，閉鎖結合は閉じ，クリステが渦巻き状の変形をきたしたミトコンドリアを認めた．脳の毛細血管内皮細胞，周囲細胞，星状膠細胞のミトコンドリアの大きさ，数ともに増大したが，神経細胞内のミトコンドリアは正常に保たれていた．グリコーゲン顆粒は，13歳時の筋肉筋原線維間，脳の毛細血管内皮細胞，神経細胞に認められ，星状膠細胞には認められなかった．これら各構成細胞におけるミトコンドリアの形態変化差異は，MELASにおける慢性乳酸性アシドーシスとATP枯渇に対応し，毛細血管内皮細胞閉鎖結合を積極的に開放する神経保護作用と考えた．