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Effects of L-arginine on the acute phase of strokes in three patients with MELAS

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The primary cause for strokelike episodes in young patients with MELAS (myopathy, encephalopathy, lactic acidosis, and strokelike episodes)—whether mitochondrial cytopathy, angiopathy, or both—remains controversial. Based on a hypothesis that strokelike episodes in MELAS are caused by segmental impairment of vasodilation in intracerebral arteries, we administered L-arginine to three patients with MELAS in the acute phase of stroke (within 1 hour of onset) and evaluated effects on clinical course, biochemical measurements, and functional cerebral hemodynamics according to ^{99m}Tc-ECD SPECT.

Patients and methods. Patients. Patient 1 was a 17-year-old woman referred to the hospital for periodic vomiting, hemiconvulsion, and short stature (below 2.7 SD). Patient 2 was an 18-year-old woman who was admitted to the hospital for generalized muscle weakness, periodic vomiting, hemiparesis, and short stature (below 1.5 SD). Patient 3 was a 15-year-old boy referred to our hospital for hemiblindness, hemiconvulsions, vomiting, and short stature (below 2.8 SD).

All patients showed extensive calcification in the basal ganglia, lactic acidosis (3.8 to 5.6 mmol/L; normal range 0.3 to 1.3) and a high lactate/pyruvate (L/P) ratio (exceeding 20). A muscle biopsy specimen showed ragged-red fibers and the percentage mutation in the A3243G of mitochondrial $tRNA^{Leu(UUR)}$ gene is 87% in Patient 1, 74% in Patient 2, and 58% in Patient 3. Growth hormone levels were low in basal secretions and were not induced by 0.5 mg/kg/dose of L-arginine loading test in all patients studied.

Methods. Patients gave informed consent, and the L-arginine study protocol was approved by the University Ethics Committee (Kurume University Institutional Review Board no. 9715). Patients had been admitted to the hospital 16 times for strokelike episodes. On these occasions, patients took part in this L-arginine vs placebo study beginning within 1 hour of onset of strokelike symptoms. Patients were administered L-arginine (0.5 g/kg/dose) as a 10% solution in nine separate strokelike episodes; a placebo (5% dextrose, 0.5 g/kg/dose) in four other episodes; and D-arginine (in a 10% solution) in the last episode. Each treatment was given IV over 15 minutes during the acute phase of stroke. The following symptoms were evaluated before and at 15 minutes, 30 minutes, and 24 hours after administration: headache (scored on a scale from 0 [no pain] to 3 [severe pain]), clinical disability (scored from 0 [no disability] to 3 [severe disability]), nausea (present or absent), vomiting (present or absent), and teichopsia (present or absent), as described elsewhere.[1] Biochemical measurements were determined including the concentrations of L-arginine, L-citrulline, pyruvate, and lactate, as well as the L/P ratio in serum or CSF. Nitric oxide metabolites (NOx) in urine were also measured. Intracranial hemodynamics were measured using ECD-SPECT (approximate total radioactivity, 740 MBq) before and after L-arginine administration.

Statistical analyses were performed Fisher's exact test for the clinical improvements, and Student's *t*-test for the biochemical measurements. The level of significance was set at $p < 0.05$.

Discussion. After administration of L-arginine, all symptoms suggesting stroke except teichopsia dramatically improved (see the table on the next page). Effects on headache, nausea, and vomiting were marked. However teichopsia remained for several days after L-arginine treatment. The reason that teichopsia remained after several days after L-arginine treatment is unclear. No adverse effects were shown. Mean arterial pressure after L-arginine treatment reached a minimum at 30 minutes after administration, coinciding with the L-citrulline peak. Lactate and pyruvate levels in serum were significantly improved at 24 hours after treatment, and were comparable to those measured during periods of well being (see the table).

At 30 minutes after L-arginine injection, uptake in the decreased regional cerebral blood flow (rCBF) in the ischemic region was improved on SPECT; however, the percent increase was less than 13% of the increase on the contralateral side. Because L-arginine did not show the responsive cerebral vasodilation during the ischemic process,[2] we cannot analyze the percent increase in rCBF accurately because the areas contained the old infarction. Luxury perfusion in the ischemic area has been reported even 4 months after a strokelike episode.[3] In this study, we wanted to prevent ischemic brain damage in the acute phase of a strokelike episode in MELAS by using L-arginine to induce vasodilation. L-Arginine is a potent donor of nitric oxide, which may reduce ischemic damage in the acute phase of focal brain ischemia by increasing CBF,[4] inhibiting postischemic leukocyte–endothelial adhesion,[5] decreasing the amount of hydroxy radical,[6] and inhibiting the potentially neuroexcitotoxic NMDA receptor.[7] However, exact mechanisms involving L-arginine and NO are not yet fully known.

Our data indicate that L-arginine therapy improved microcirculation and reduced tissue injury from ischemia; therefore, it represents a potential new therapy for use in the acute phase of strokelike episodes in MELAS.

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Table Effects of L-arginine

Symptoms/measurements	Before administration	After administration		
		15 min	30 min	24 h
Clinical symptoms in three patients with MELAS*				
Headache (improvement from score 3/2 to 1/0)				
L-Arginine	0/9	0/9	4/9†	5/9†
Placebo¶	0/7	0/7	1/7	1/7
Clinical disability (improvement from score 3/2 to 1/0)				
L-Arginine	0/9	0/9	4/9†	5/9†
Placebo¶	0/7	0/7	0/7	0/7
Nausea				
L-Arginine	0/9	0/9	4/9†	4/9†
Placebo¶	0/7	0/7	0/7	1/7
Vomiting				
L-Arginine	3/9	3/9	5/9†	6/9†
Placebo¶	0/7	0/7	0/7	1/7
Teichopsia				
L-Arginine	0/9	0/9	0/9	1/9
Placebo¶	0/7	0/7	0/7	0/7
Biochemical measurements, mmol/L, in Patient 1 (normal values)‡				
L-Arginine, blood (0.10 ± 0.04)	0.06 ± 0.01	10.6 ± 0.05§	4.7 ± 0.01§	0.08 ± 0.01
L-Citrulline, blood (0.02 ± 0.01)	0.08 ± 0.01	0.12 ± 0.01§	0.18 ± 0.01§	0.09 ± 0.01
Pyruvate, blood (0.10 ± 0.02)	0.22 ± 0.02	0.21 ± 0.01	0.20 ± 0.04	0.15 ± 0.02§
Lactate, blood (1.02 ± 0.08)	4.46 ± 1.02	6.48 ± 0.79§	7.28 ± 0.55§	2.85 ± 0.47§
L/P ratio, blood	17.8 ± 0.58	25.4 ± 7.97	28.0 ± 8.15	16.2 ± 1.03
Pyruvate, CSF	0.32	ND	ND	0.22
Lactate, CSF	4.86	ND	ND	3.22

* Numbers indicate the number of occasions when improvement was seen relative to the total number of episodes.
† $p < 0.05$ by Fisher's exact test.
‡ Numbers indicate mean ± SD in four separate occasions of strokelike episodes.
§ $p < 0.05$ by one-tailed student's t-test.
¶ 5% dextrose (0.5 g/kg/dose) in four other episodes and D-arginine in three episodes.

ND = not determined.

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Increased mitochondrial processing intermediates associated with three tRNA$^{Leu(UUR)}$ gene mutations

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Abstract

Accumulation of RNA 19 has been associated with mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes. We analyzed total RNA in muscle specimens from six patients who had one of three pathogenetic point mutations in the mitochondrial tRNA$^{Leu(UUR)}$ gene, including A3243G, T3271C, and T3303C. Mitochondrial processing intermediates were identified and quantitated by Northern blotting. The percentage of DNA with the mutation also was determined in each patient. The intermediate (RNA 19) was significantly increased in all patients. The proportion of mutation-carrying RNA in processing intermediates was always higher than in the DNA fraction, suggesting that these mutations may have dominant-negative effects on mitochondrial RNA processing events at the tRNA$^{Leu(UUR)}$ gene boundary.

Keywords: Mitochondrial DNA mutation; RNA; Dominant-negative effect

1. Introduction

Patients with a mutation at the tRNA$^{Leu(UUR)}$ gene boundary show varied clinical manifestations such as mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS), encephalomyopathy, Leigh disease, maternally inherited diabetes mellitus, chronic external ophthalmoplegia, and cardiomyopathy. Understanding genotype-phenotype correlations in the MELAS patients will require much investigation of complex and incompletely delineated pathogenetic mechanisms. Mitochondrial processing intermediates (RNA 19), which consist of RNA species of the 16S rRNA/tRNA$^{Leu(UUR)}$/ND 1 genes, were originally discovered in the transmitochondrial cell lines containing the A3243G mutation associated with MELAS [1]. RNA 19 has also been detected in cybrid cells containing a mutation at position T3271C [2] or C3256T [3] in the tRNA$^{Leu(UUR)}$ gene, and abnormal accumulation of RNA 19 has also been detected in muscle cells and fibroblasts from a patient with a T3302C mutation [4], and in muscle cells with an A3243G mutation in the tRNA$^{Leu(UUR)}$ gene [5]. Such findings imply that abnormal mitochondrial RNA processing contributes to the pathogenesis of disease caused by tRNA$^{Leu(UUR)}$ gene mutations. In 1998 we found that abnormal accumulation of RNA 19 caused dominant-negative effects in association with an A3243G point mutation in the mitochondrial tRNA$^{Leu(UUR)}$ gene [6].

On investigating the molecular pathogenetic mechanisms underlying MELAS, interpretation of observations in cultured cells such as those above presented a major problem because the experiments were carried out in immortalized cell lines, usually derived from tumors. Characteristics of such aneuploidy are not constant, even for a given cell line. To avoid problems of interpretation in connection with aneuploidy, we used tissue specimens from patients to determine genotype-phenotype relationships associated with point mutations in the tRNA$^{Leu(UUR)}$ gene. We analyzed steady-state levels and percentages of processing intermediates in patients having four different clinical phenotypes including Leigh encephalomyelopathy, MELAS, progressive external ophthalmoplegia (PEO), and mitochondrial cardiomyopathy. Each patient had one of three MELAS related point mutations in this region (A3243G, T3271C, or T3303C). Our aim was to determine the biologic significance of gene processing at the mitochondrial tRNA$^{Leu(UUR)}$ boundary, and to examine whether tissue-specific mitochondrial RNA processing might be responsible for the clinical heterogeneity associated with point mutations in the mitochondrial tRNA$^{Leu(UUR)}$ gene.

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Table 1
Clinical and genetic findings in patients[a]

Patient	1	2	3	4	5	6
Phenotype	Leigh	MELAS	MELAS	PEO	MELAS	MMP
Onset/biopsy (y)	1/4	3/13	23/26	32/42	18/18	0.4/0.5
Outcome (y)	death(9)	alive	alive	alive	alive	death (0.6)
RRF (%)	68	5	6	0.5	3	59
SSVs	+	+	+	−	+	+
Point mutation	A3243G	A3243G	A3243G	A3243G	T3271C	T3303C
% RNA 19 in the total ND 1 signal (control 3.5 ± 0.8)						
	21.6	14.1	15.5	17.2	12.6	27.9
% Mutation in muscle homogenate in DNA						
	92	87	74	33	65	67
% Mutation in RNA 19 fraction (control, 0%)						
	96	92	86	89	87	96

[a] Key: Leigh, Leigh encephalomyelopathy; MELAS, mitochondrial myopathy, encephalopathy, lactic acidosis and strokelike episodes; PEO, progressive external ophthalmoplegia; MDM, mitochondrial diabetes mellitus; MMP, mitochondrial myopathy; RRF, ragged-red fibers; and SSVs, strongly succinate dehydrogenase-hyperreactive vessels. The control value for % RNA 19 is expressed as the mean ± SD of the percent of the total hybridization signal ($N = 8$).

2. Patients and methods

2.1. Patients

Clinical and genetic findings in the patients in this study are summarized in Table 1. Patients 1–4 had an A3243G mutation in the mitochondrial $tRNA^{Leu(UUR)}$ gene, and were described as patients 1–4 in a previous report [7]. A detailed clinical summary of patient 6 also has been reported [8]. All patients gave informed consent for participation in this study, which was approved by the local ethics committee (Kurume University IRB#9715).

2.2. DNA and RNA analysis

Total DNA was extracted according to an established protocol [1]. Previously described methods were used to screen for A3243G [1], T3271C [2], and T3303C [8] mutations. Total RNA was isolated and analyzed by a method described previously [1]. RNA hybridization signals were quantitated using a BAS 2000 II Bioimage Analyzer (Fujix, Tokyo, Japan).

2.3. Mutation analysis of mitochondrial RNA processing intermediates

Mitochondrial RNA processing intermediates including RNA 19 were excised individually from gels after electrophoresis to eliminate any contaminating DNAs and RNAs. RNA was extracted using a gene matrix method (Gene Clean, Bio 101, CA) and then digested with RNAse-free DNAse I (Takara Biomedical, Tokyo, Japan). Using the same set of primers used for DNA analysis, the RNA processing intermediates were amplified from the $tRNA^{Leu(UUR)}$ fraction using a 'hot start' program and the thermostable rTth reverse transcriptase RNA polymerase chain reaction (PCR) system (Perkin Elmer Cetus, Norwalk, CT). RNA (100 ng) was amplified by rTth DNA polymerase-PCR, for 1 cycle of 2 min at 94°C, followed by 35 cycles of 1 min at 95°C, and 1 min at 60°C, and then a final cycle at 60°C for 7 min. To this PCR product were added 10 mCi of [α-^{32}P] dATP (3000 Ci/mmol), and 2.5 U of *Taq* polymerase; this mixture was incubated for 2 min at 94°C, 1 min at 55°C, and 10 min at 72°C. The resulting products were digested as in the DNA analyzes. DNA restriction fragments were measured quantitatively using the same procedure described above.

3. Results and discussion

We analyzed steady-state levels and processing of RNAs derived from the region of the $tRNA^{Leu(UUR)}$ gene boundary in autopsy and biopsy tissues samples from six patients harboring mutations of A3243G, T3271C, or C3303T in the $tRNA^{Leu(UUR)}$ gene. Steady-state levels of processing intermediates in muscle from patients who had a point mutation in the $tRNA^{Leu(UUR)}$ gene were significantly higher than those seen in normal tissues (Fig. 1 and Table 1). This patient showed 96% of the RNA 19 fraction contained the mutation in patient 6 with T3303C, the greatest accumulation of RNA 19 (eight times the control total ND 1 signal, and 1.7 times the control total $tRNA^{Leu(UUR)}$ signal). Sixty-seven percent of DNA from muscle homogenate contained the T3303C mutation (Fig. 2). Patient 1, who showed the second highest accumulation of RNA 19 (21.6% of the total ND 1 signal), also had the highest percentage of mutation in DNA from muscle homogenate (92%) among patients with an A3243G. Both patients died during the first decade of life.

In controls, the highest percentage of RNA 19 was present in the brain (Fig. 1), which suggests an important role for this

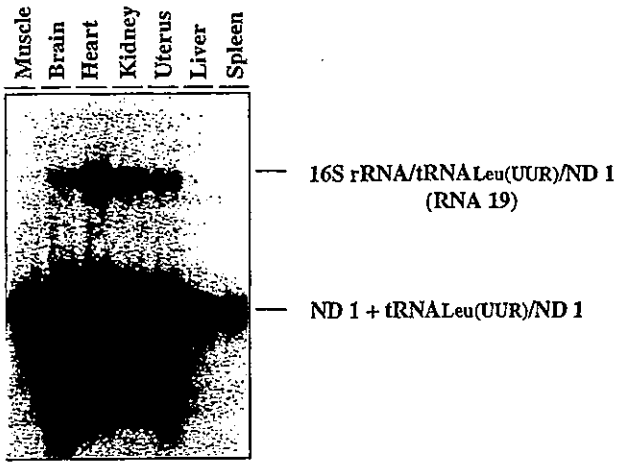

Fig. 1. Northern (RNA) hybridization analysis of normal human tissues. Autoradiograms of the hybridization blot of total RNA isolated from various tissues from a normal individual are shown. The two species hybridizing to the ND 1 probe are indicated at the right. One is approximately 2600 nucleotides (nt) in size and corresponds to RNA 19, while the other is approximately 950 nt and corresponds to ND 1 and to $tRNA^{Leu(UUR)}$ plus ND 1. RNA 19 also was detected by the 16S rRNA probe and by the $tRNA^{Leu(UUR)}$ probe (data not shown).

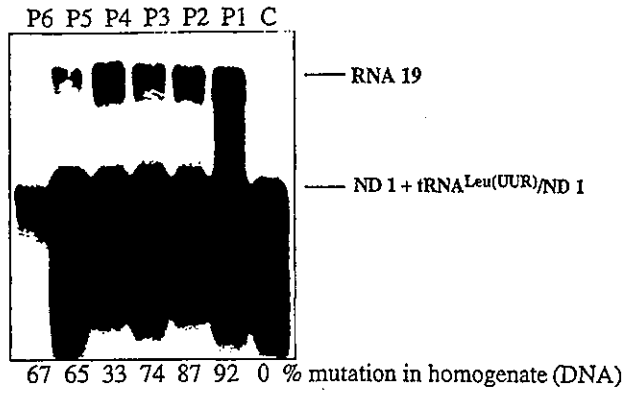

Fig. 2. Northern (RNA) hybridization analysis of patient tissues. Autoradiograms of hybridization blots of total RNA isolated from muscle biopsy specimens from six patients are shown. The two species hybridizing to the ND 1 probe are indicated at the right. One approximately 2600 nt in size, corresponds to RNA 19, while the other, at approximately 950 nt, corresponds to ND 1 and $tRNA^{Leu(UUR)}$ plus ND 1. RNA 19 also was detected with the 16S rRNA probe (data not shown). Steady-state levels of each processing intermediate are shown in Table 1, and are expressed as a percentage of the total ND 1 or $tRNA^{Leu(UUR)}$ signals.

intermediate in neurologic aspects of MELAS such as stroke-like episodes. In our study, steady-state levels of RNA 19 in human tissues showed variation possibly resulting from differences in energy dependency, tissue-specific factors or mitochondrial RNA processing capacity of the cells; these variables may be controlled by the nuclear genome. Steady-state levels of processing intermediates differ between tissues, and a strong inverse correlation can be demonstrated between the level of RNA 19 per cell and the rate of oxygen consumption in cybrid cell lines in vivo [1]. Tissues such as brain, heart, muscle, and pancreatic β-cells may lose some tolerance to respiratory insufficiency when processing intermediates accumulate. Certain mutations in the human mitochondrial $tRNA^{Leu(UUR)}$ gene have been reported to interfere directly with efficient processing of the tRNA precursor in vitro [9]. Our patients 1 and 6, who died in their first decade, showed higher accumulations of RNA 19 (greater than 27% of the total ND 1 signal and more than 46% of the total $tRNA^{Leu(UUR)}$ signal) than our other patients who survived. Although the T3303C mutation showed no marked effect on processing in vitro [9], a severe processing abnormality was observed in patient tissues. The degree of RNA 19 accumulation does not always correlate with the percentage of mutation in a DNA-based analysis; this was especially true in patient 6. Accumulation may be influenced by the location of the mutation within the $tRNA^{Leu(UUR)}$ gene, and also the processing capacity of individual tissues. The processing intermediates with normal segments encoding runes might be incorporated into ribosome and render them functionally deficient 'ribosome-stalling' [10]. Even low levels of processing intermediates could exert strong inhibitory effects on the mitochondrial translation system, especially with the longer translation products seen in the mutant cybrids [2]. While we know little concerning the biologic significance of the processing intermediates, we might speculate that intermediates such as RNA 19 could be involved in controlling respiratory chain enzyme activity, or could serve as a messenger by which mtDNA communicates with nuclear DNA in disease states.

Combined with our previous observations [6], the present findings indicate that percentages of mutations in the processing intermediates are always higher than percentages in mtDNA. This suggests that processing intermediates that contain mutations, including RNA 19, may be more difficult to process than wild-type segments. However, no qualitative differences in the processing of the 5′- or 3′-ends were noted between cybrids with mutant DNA and cybrids with wild-type DNA. In the present study, we analyzed two additional point mutations in the $tRNA^{Leu(UUR)}$ gene, with results similar to those seen with A3243G. Our data indicate that dominant-negative effects in mitochondrial RNA processing can occur when a point mutation is present at the $tRNA^{Leu(UUR)}$ gene boundary. However, we have no definitive evidence demonstrating more rapid accumulation of mutant RNA 19 in any specific tissue. RNA 19 elevations have not been observed in myoclonus epilepsy with ragged-red fibers or in the Kearns-Sayre syndrome cybrid system. On the other hand, accumulation of RNA 19 has been reported in patients with a novel point mutation in the mitochondrial $tRNA^{Leu(UUR)}$ gene. We therefore suspect that accumulation of RNA 19 may be a specific consequence of mitochondrial $tRNA^{Leu(UUR)}$ mutations.

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臨床編 II

ミトコンドリア病(狭義) 欠損複合体別分類と臨床

複合体I

Respiratory chain enzyme complex I

古賀靖敏　植木 勲

Key words: 電子伝達系酵素複合体I, MELAS, ミトコンドリアDNA, 核DNA, 代謝性アシドーシス

1. 概念・定義

複合体I(NADH-ubiquinone oxidoreductase, EC: 1.6.5.3)は，ミトコンドリア呼吸鎖の電子伝達系複合体の最初の酵素であり，NADHから電子を受け取りコエンザイムQに電子を渡す役割を担う．この反応過程で，マトリックスから膜間腔側へプロトンをくみ出す．

この酵素は，少なくとも43個の異なるサブユニットからなる酵素複合体であり，電子伝達系酵素の中で最も大きい．ミトコンドリアDNAにコードされた7個のサブユニットと，核DNAにコードされた少なくとも36個のサブユニット(flavin mononucleotideや8つのnon-hem iron-sulfur clusterを含む幾つかの非蛋白成分も含む)からなる酵素複合体で構成され(ミトコンドリアDNAと核DNAの両支配)，分子集合により活性を獲得する．

核DNA由来のサブユニットの多くは，N末端に20-80個のアミノ酸からなるプレ配列(ミトコンドリアターゲティングシグナル)をもつ前駆体蛋白として細胞質で合成される．このプレ配列を認識した細胞質シャペロン系(Hsp70シャペロン)により，細胞内小器官であるミトコンドリア外膜のインポート受容体に輸送される．

ミトコンドリア外膜には，Tom(translocator of outer membrane)複合体，内膜にはTim(translocator of inner membrane)複合体と呼ばれる蛋白質輸送装置が存在し，前駆体蛋白は外膜から内膜へ転送される．マトリックスに達した前駆体蛋白は，MPP(mitochondrial processing peptidase)もしくはMIP(mitochondrial intermediate peptidase)の働きにより切断除去され，成熟酵素蛋白となりミトコンドリア内膜で他のサブユニットとともに分子集合(assembly)を形成する．

複合体Iの活性が低下する病態が複合体I欠損症である．多くのミトコンドリア病，その他の症候群で本酵素の活性低下が報告されている[1]．

2. 分　　類

遺伝子異常を中心にした複合体I欠損症の分類を，表1に示す．

電子伝達系酵素異常に共通する事柄として，複合体Iを構成する酵素蛋白のいずれかの遺伝的異常が病態の本質(一次的異常)であっても，病期が進行すると他の電子伝達系酵素複合体の二次的異常を伴うことが多く，酵素生化学的には明確に分別できない場合も多く存在する．また，本質的にはミトコンドリア病と考えられな

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表1 複合体I欠損症の分類

1. 遺伝子異常による複合体I単独欠損症
   a. 核DNAにコードされた複合体Iサブユニットの遺伝子異常
      1) NDUFA1(NADH-ubiquinone oxidoreductase 1 α subcomplex 1)
      2) NDUFA8(NADH-ubiquinone oxidoreductase 1 α subcomplex 8)
      3) NDUFB6(NADH-ubiquinone oxidoreductase β subcomplex 6)
      4) NDUFS1(NADH-ubiquinone oxidoreductase Fe-S protein 1)
      5) NDUFS2(NADH-ubiquinone oxidoreductase Fe-S protein 2)
      6) NDUFS3(NADH-ubiquinone oxidoreductase Fe-S protein 3)
      7) NDUFS4(NADH-ubiquinone oxidoreductase Fe-S protein 4)
      8) NDUFS5(NADH-ubiquinone oxidoreductase Fe-S protein 5)
      9) NDUFS7(NADH-ubiquinone oxidoreductase Fe-S protein 7)
      10) NDUFS8(NADH-ubiquinone oxidoreductase Fe-S protein 8)
      11) NDUFV1(NADH-ubiquinone oxidoreductase flavoprotein 1)
      12) NDUFV2(NADH-ubiquinone oxidoreductase flavoprotein 2)
   b. ミトコンドリアDNAにコードされた複合体Iサブユニットの遺伝子異常
      1) MTND1(mitochondrial complex I subunit 1)
      2) MTND4(mitochondrial complex I subunit 4)
      3) MTND6(mitochondrial complex I subunit 6)
2. 他の電子伝達系酵素複合体欠損を伴う複合体I欠損症
   a. ミトコンドリアtRNA遺伝子の異常
      1) MTTL1(mitochondrial tRNALeu(UUR) gene mutation)
         MELAS(mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes)を含む
      2) MTTK(mitochondrial tRNALys gene mutation)
         MERRF(myoclonus epilepsy with ragged-red fibers)を含む
      3) MTTG(mitochondrial tRNAGly gene mutation)
   b. ミトコンドリアにコードされた酵素蛋白遺伝子の異常
      1) MTATP6(mitochondrial ATP synthase 6)
         NARP(neurogenic muscle weakness, ataxia, retinitis pigmentosa), 母系遺伝を示すLeigh脳症
      2) LHON(Leber hereditary optic neuropathy)
      3) MTCO1(mitochondrial complex IV subunit 1)
   c. ミトコンドリアDNAの大欠失, duplication, deletionによる異常
      1) Kearns-Sayre症候群(KSS)
      2) chronic progressive external ophthalmoplegia (CPEO)
      3) mitochondrial DNA depletion
   d. 核でコードされた酵素蛋白遺伝子の異常
      1) UQCRC1(uniquinol-cytochrome c reductase core protein 1)
      2) SDHA(succinate dehydrogenase complex, subunit A, flavoprotein)
      3) COX4(cytochrome c oxidase subunit 4)
      4) COX10(cytochrome c oxidase assembly protein COX10)
      5) COX17(cytochrome c oxidase assembly protein COX17)
      6) ピルビン酸脱水素酵素複合体E1α欠損症(PDHA1)
      7) メープルシロップ尿症
3. 他の症候群に伴う複合体I欠損症
   a. Parkinson病
   b. Alzheimer病
   c. Leigh脳症
   d. MENKES症候群
   e. Alpers progressive sclerosing poliodystrophy
   f. Barth症候群
   g. Friedreich ataxia
   h. 遺伝性痙性対麻痺
   i. MNGIE(mitochondrial myopathy, peripheral neuropathy, gastrointestinal and encephalopathy disease)
   j. Mohr-Tranebjaerg症候群(DFN1)
   k. Pearson症候群
   l. syndromic forms of sensorineural hearing loss
   m. Wolfram症候群

い種々の病気，病態で，複合体I酵素欠損が報告されている[2]．

多くの複合体I酵素欠損が報告される理由として，①構成するサブユニット数が最も多い，②核DNAとミトコンドリアDNAの二重支配であり，その酵素活性発現に蛋白の転送および分子集合などの複雑な機構が関与する，③鉄硫黄画分が検体の保存状態によっては不安定で

表2 複合体I欠損症の症候

臓器/システム	症候/徴候
1. 中枢神経系	体幹の低緊張，首座りの遅れ，精神発達遅滞，知的退行，脳神経障害，痙性四肢麻痺，末梢神経障害，小脳失調，亜急性脳幹壊死，白質ジストロフィー，ポリオジストロフィー，ミオクローヌス，てんかん，めまいを伴う片頭痛発作，脳卒中発作，呼吸不整(無呼吸発作)
2. 胎内発育	成長障害，子宮内発育不全
3. 骨格筋	筋力低下，筋痛，筋強剛，易疲労性，反復性ミオグロビン尿症
4. 肝 臓	肝腫大，肝障害，肝不全
5. 心 臓	心筋症(肥大型，拡張型)，心刺激伝導ブロック
6. 腎 臓	近位尿細管性アシドーシス，間質性腎炎，ネフローゼ症候群，腎不全，溶血性尿毒症症候群
7. 消化器	慢性下痢，絨毛萎縮，反復性嘔吐，食思不振症，慢性的な偽腸閉塞症，膵外分泌不全，十二指腸閉鎖，慢性消化吸収障害
8. 造血組織	貧血，好中球減少症，血小板減少症，骨髄異形成，赤芽球低形成
9. 内分泌系	低身長，骨年齢の遅延，GH不応性IgF1欠損症，反復性低血糖症，糖尿病(IDDM，NIDDM)，甲状腺機能低下症，副甲状腺機能低下症，ACTH欠損症
10. 聴 覚	感音性難聴，聴力低下，聴覚毒性
11. 視 覚	眼瞼下垂，複視，進行性外眼筋麻痺，眼球運動の制限，白内障，視神経萎縮，網膜色素変性症
12. 外表奇形	小頭症，丸顔，前頭部突出，鼻根部扁平，耳介低位，頸部短縮，小さな手，爪の低形成
13. 皮 膚	皮膚の脂肪組織増加，日光照射部の色素沈着，縮れ毛
14. 検査異常	ケトアシドーシス性昏睡，代謝性アシドーシス，低血糖，高乳酸血症，高アラニン血症，低シトルリン血症

容易に失活しやすい，④電子伝達系酵素欠損症に共通する問題として，酵素診断では一次的な異常と二次的な異常が混在する可能性があること(検体の長期保存，病期が進行した状態での検索)などが関係していると思われる．

したがって，個々の症例における複合体I欠損症の病因を評価する場合，上記に述べた注意点を考慮する必要がある．

3. 病因論的事項

複合体Iがミトコンドリアと核の両支配であることより，本症の遺伝形式としてメンデル遺伝(常染色体性劣性，X連鎖性劣性)および母系遺伝のすべてを考慮しなければならない．

複合体I欠損症が核DNA遺伝子の異常で発症する場合，生後早期に呼吸不全，重症の代謝性アシドーシス，精神運動発達遅滞，フロッピーインファントで見つかり，比較的早期に死亡する乳児重症型が多い[3]．複合体I+III，II+IIIでの活性低下が同時にみられた場合，コエンザイムQの異常を考える[4]．複合体I，II，IIIおよびアコニターゼの合併欠損であれば，iron-sulfur proteinの異常を疑う[5]．複合体I，II，III，IVの合併欠損であれば，核成分のミトコンドリアへの移送障害もしくは，ミトコンドリアDNAのdepletionを疑う[2]．複合体IとIVの合併欠損がみられた場合，ミトコンドリアDNAの点変異，欠失などを疑う[2]．

特に，ミトコンドリアDNAの点変異(MELAS)では，本酵素の低下が多く報告されている[6]．これらの生化学的検査結果は，病期との関係から二次的変化をも含むオーバーオールの結果であり，その判断には臨床症状および経過の把握が不可欠である．

4. 病態(症候論と検査成績)

電子伝達系酵素が細胞の生命活動の維持に必須であることから，その異常はあらゆる臨床症状の組み合わせを来し得る．本症でみられる症候を表2に示す[7]．

電子伝達系酵素欠損症の特徴は，症状が進行することである．同じ患者に2年以上の間隔で2回以上筋生検を行った場合，筋病理学的にも，酵素生化学的にも症状の重症度が進行すること

が証明された[8]．症状が進行しエネルギー不全状態が進み，蛋白合成が全般的に阻害された時期に生化学的検索を行っても，一次的異常が何かを特定することは難しい[9]．また，全身的にみられる症候も，時間的に小変動を繰り返しながら次第に悪化し，他の症候が加わっていくことが多く経験される．

5. 診断と鑑別診断

診断には，信頼できる検体を用いることが基本である．電子伝達系酵素の一員である複合体Iの活性は，ロテノン感受性を有する活性部分である．検索材料に関して，電子伝達系酵素活性に依存度が低い末梢血白血球を用いては，信頼できる結果が得られにくい．全NADH酸化反応におけるロテノン依存性活性は，末梢血単核球分画の細胞ホモジネートを使用した場合，3％以下にまで低下する(mg蛋白当たりの活性値)．

現在最も信頼できる複合体I活性の測定は，新鮮な生検骨格筋検体からミトコンドリア分画を分離精製し，NADHなどを基質としたロテノン依存性酸素消費能の測定(oxograph)，NADH dehydrogenase，NADHからcytochrome Cまで鉄硫黄蛋白画分を含めた酵素活性測定を同時に行うことである．この場合，細胞質の夾雑蛋白の混入の指標として，citrate synthaseなどに対する比活性で補正する方法もとられている．その評価には，様々な因子が関与するため，経験ある施設で行うことが望ましい．

6. 治療と予後

今までにコエンザイムQ_{10}，イデベノン，メナジオン，リボフラビン，コハク酸，コルチコステロイド，カルニチン，クレアチン，ビタミンKおよびC，ジクロロ酢酸，カルジオクロームなどが投与されてきた．最近，著者らは，急性期のミトコンドリア脳卒中(MELAS)にL-アルギニンを投与し，卒中様症状の消失，SPECTによる脳血流の改善を報告した[10]．MELASにおける発作急性期の治療として期待される．現在，本症の基本病態改善に対する特異的な治療薬はなく，以上に述べた対症療法が重要である．

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臨床編 II

ミトコンドリア病(狭義) 欠損複合体別分類と臨床

複合体II

Complex II

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Key words: 複合体II, コハク酸脱水素酵素, コエンザイムQ, 鉄-硫黄蛋白, SDH染色

1. 概念・定義

複合体II(succinate-ubiquinone oxidoreductase, EC: 1.3.5.1)は, ミトコンドリア呼吸鎖の酵素であり, 電子伝達系酵素複合体の中で, 唯一, 核DNAのみでコードされた5個のサブユニットからなる酵素複合体で構成される. その主な構成成分であるコハク酸脱水素酵素(succinate dehydrogenase: SDH, EC: 1.3.99.1)は, 4つのサブユニットからなり, catalytic coreであるflavoprotein(Fp: SDHA), iron-sulfur protein subunit(IP: SDHB)とそれらをミトコンドリア内膜につなぎ止める役割を果たす2つのintegral membrane protein subunits(SDHC, SDHD)で構成される(図1)[1].

このSDHはTCAサイクルのkey step enzymeでもあり, succinateを水酸化しfumarateを生成し, 同時に電子を受け取りコエンザイムQに渡す役割を担う. サブユニットは, N末端にプレ配列(ミトコンドリアターゲティングシグナル)をもつ前駆体蛋白として細胞質で合成される. その後, 細胞質シャペロン系(Hsp70シャペロン)により, 細胞内小器官であるミトコンドリア外膜のインポート受容体に輸送される. この複合体IIの活性低下を来す病態を複合体II欠損症と呼ぶ. 多くのミトコンドリア病, その他の症候群で本酵素の活性低下が報告されている.

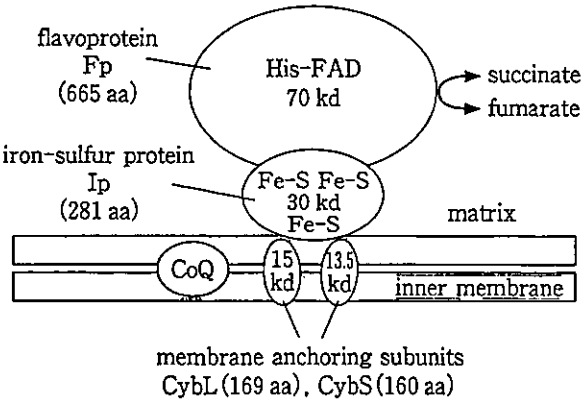

図1 複合体IIの構造

2. 分　　類

今までに報告された複合体II欠損症を, 表1に示す. 複合体IIの構成遺伝子が単離されるまでは, 酵素生化学的分類により3群に大別された[1]. 複合体IIが単独に欠損する場合, 他の電子伝達系複合体欠損を合併する場合, その他の疾患に合併する場合が報告されている. 複合体II単独欠損の症例は, 更にSDH欠損とそれ以後の電子の授受に異常がある場合とに分けられる. SDH欠損例は, 骨格筋生検で筋組織化学的に証明される場合があり, びまん性に染色性の低

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