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# The treatment of mitochondrial myopathies and encephalomyopathies

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**Abstract**

This paper briefly summarizes the results of a long-term, open pharmacotherapy trial in 16 patients with well-characterized mitochondrial disease. Outcome measures included repeated clinical evaluation, $^{31}$P-NMR spectroscopy and near-infrared spectroscopy. Treated patients appeared to survive longer with less functional disability and medical complications than typically seen in clinical practice.

*Keywords:* Mitochondrial disease; Mitochondrial myopathy; Treatment; Pharmacotherapy

## 1. Introduction

Mitochondrial diseases are degenerative disorders which result from progressive decline in the ability to supply cellular energy requirements. The mitochondrial diseases include myopathies, encephalopathies and encephalomyopathies, and are characterized by clinical, biochemical and genetic heterogeneity. From a clinical perspective, the phenotype can vary extensively, and depends primarily on the severity of mitochondrial dysfunction, the patient's age at clinical presentation, and the tissues primarily involved in the disease. Progressive multisystem involvement is common, and those tissues requiring a large supply of ATP (e.g., central nervous system, muscle, retina, liver and kidney) are most susceptible to clinically apparent dysfunction (Table 1).

The most commonly encountered mitochondrial diseases in clinical practice are mitochondrial myopathies. In these disorders, most patients present during the adult years with progressive ptosis, external ophthalmoplegia, muscle weakness and fatiguability. Multisystem dysfunction consisting of progressive neurosensory hearing loss, pigmentary retinal deterioration and peripheral neuropathy are not uncommon. The majority of these patients remain productive for many years and die from causes unrelated to their primary disease, i.e., ischemic cardiac or cerebrovascular disease. Kearns–Sayre syndrome (KSS) is a mitochondrial encephalomyopathy which typically presents in the pediatric age group and is characterized by progressive ptosis, external ophthalmoplegia, cardiac conduction defects, ataxia, short stature, pigmentary retinopathy, endocrinopathies, progressive dementia, elevated cerebrospinal fluid protein and neurosensory hearing loss. Defects of cardiac conduction are progressive in nature and may result in sudden death; placement of a prophylactic cardiac pacemaker is frequently required. The majority of patients are significantly disabled by their disease, experience a progressive decline in clinical status and die from causes directly related to their disease, e.g., cardiac failure. Mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS) is characterized by non-vascular stroke-like episodes, migrainous headaches, lactic acidosis, epilepsy and progressive dementia. The clinical course is unpredictable, patients become progressively disabled in a step-wise fashion and the prognosis is characteristically poor. Most patients succumb to the disease or a complication directly related to the disease in a short period of time. Mitochondrial encephalomyopathy with ragged red fibers (MERRF) is an infrequently encountered mitochondrial encephalomyopathy characterized by prominent myoclonus, epilepsy, cerebellar dysfunction and progressive dementia. Gastrointestinal dysfunction is not uncommon. The majority of these patients die from status epilepticus or complications related to their seizure disorder.

Although new mitochondrial syndromes are frequently described, mitochondrial myopathy, KSS, MELAS and MERRF are of most significance to the clinician due to the fact that they are more commonly encountered than other

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Table 1
Multisystem involvement in mitochondrial disease

| System | Clinical/laboratory features |
|---|---|
| Central nervous system | seizures, dementia, neurosensory hearing loss, myoclonus, cerebellar dysfunction, stroke-like episodes |
| Muscle | ocular myopathy (ptosis, external ophthalmoplegia), proximal myopathy with fatiguability, cardiomyopathy |
| Retina | pigmentary retinopathy |
| Liver | impaired hepatic function |
| Kidney | renal tubular dysfunction |
| Other | neuropathy, impaired intestinal absorption, endocrinopathy (glucose intolerance, hormonal insufficiency) |

mitochondrial diseases. Several recent clinical reviews of mitochondrial diseases are recommended for the interested reader [1–3]. Significant advances have been made in mitochondrial genetics and several disease specific mitochondrial DNA mutations have been identified [4,5].

Growing recognition of mitochondrial diseases and their clinical diagnosis have necessitated the search for effective therapy. Despite the poor therapeutic potential associated with the term 'degenerative disease', understanding of the pathophysiology of mitochondrial disease has facilitated efforts to develop effective clinical therapy. It is now recognized that a significant pathophysiologic consequence of mitochondrial dysfunction includes failure of energy-dependent ionic balance, with subsequent increase in intracellular calcium content and initiation of lipolysis and proteolysis [6–8]. Accumulation of lactic acid and partial reduction of respiratory activities leads to the production of reactive oxygen species. These pathophysiologic consequences have prompted the use of antioxidants, electron transfer mediators (which by-pass the inhibitory site), enzyme cofactors and agents which block calcium-activated degrading pathways in clinical practice [9–14]. Large double-blind, clinical trials of potentially beneficial compounds have been hindered by the rarity of all of the mitochondrial diseases, their heterogeneous nature, the unpredictable nature of the clinical course, and the lack of reliable clinical outcome measures.

In this paper, the results of a long-term pharmacotherapy trial in 16 patients with well-characterized mitochondrial myopathy and encephalomyopathy are presented.

## 2. Clinical pharmacotherapy trial

### 2.1. Patient selection

Sixteen patients were selected from a large referral population of patients with degenerative mitochondrial disease. Those patients with mitochondrial myopathy, KSS, MELAS or MERRF who lived within a distance which would permit frequent clinical follow-up, were included in the study. The patient population is characterized in Table 2. There were 4 patients with a mitochondrial myopathy, 4 with KSS, 6 with MELAS and 2 with MERRF. Patients ranged in age from 7 to 59 years, (6 were pediatric). There were 7 male patients and 9 females.

Table 2
Clinical characteristics of study population

| Patient # | I.D. | Clinical syndrome | Sex | Age at onset of treatment (y) |
|---|---|---|---|---|
| 1. | P.R. | mitochondrial myopathy | M | 49 |
| 2. | V.I. | mitochondrial myopathy | F | 59 |
| 3. | C.M. | mitochondrial myopathy | F | 51 |
| 4. | H.M. | mitochondrial myopathy | F | 54 |
| 5. | S.J. | KSS | F | 7 |
| 6. | Y.M. | KSS | M | 23 |
| 7. | D.C. | KSS | F | 25 |
| 8. | M.R. | KSS | M | 19 |
| 9. | B.M. | MELAS | M | 34 |
| 10. | B.L. | MELAS | F | 31 |
| 11. | M.L. | MELAS | M | 19 |
| 12. | A.M. | MELAS | F | 31 |
| 13. | K.B. | MELAS | F | 33 |
| 14. | F.T. | MELAS | M | 15 |
| 15. | K.J. | MERRF | M | 19 |
| 16. | W.P. | MERRF | F | 17 |

*2.2. Biochemical studies of isolated skeletal muscle mitochondria and identification of sites(s) of impairment along the respiratory chain*

Thirteen of the patients underwent muscle biopsy for isolation and analysis of fresh skeletal muscle mitochondria. All 16 patients had prior muscle biopsies performed which revealed histochemical evidence of mitochondrial disease before referral to our center. Mitochondrial preparations were isolated and characterized as described by Makinen and Lee [15] and Lee et al. [16,17]. The respiratory and phosphorylation activities of fresh, isolated skeletal muscle mitochondria from patients with mitochondrial myopathy are briefly summarized in Table 3. All patients (#1–4) had decreased State 3 rates with both pyruvate + malate and succinate (+rotenone) as substrates, although the rates with pyruvate + malate were more significantly impaired. However, with the exception of patient #3 the capacities of energy coupling as reflected by the respiratory control index (RCI), and efficiencies of phosphorylation (ADP/O) were within the normal range. Part of these studies have been briefly communicated [18]. The data from the four patients with KSS (#5–8) are shown in Table 3. In all 4 patients the State 3 respiratory rates with NAD-linked substrate and succinate (+rotenone) were severely impaired. The cytochrome oxidase activity (data not shown) was also slower than that of controls, but was considerably faster than the rate with either succinate or NAD-linked substrates [19]. This indicated that the depressed respiratory rates with succinate and NAD-linked substrates could not be due to impairment of cytochrome oxidase. Cytochrome analyses revealed a severe deficiency of cytochrome $a + a_3$ ($\approx$ 30% of control) and an excess of $c$ cytochrome ($\approx$ 130% of control). Analyses of the steady state reduction kinetics revealed the rate-limiting step in the transfer of electrons from reduced substrates to oxygen was on the path between cytochrome $c$ and cytochrome oxidase [19]. The data obtained from fresh intact skeletal

Table 3
Respiratory and phosphorylating activities of isolated skeletal muscle mitochondria in patients with mitochondrial myopathy, KSS, MELAS and MERRF

| Substrates | Respiratory rate (nAtoms O/min per mg prot.) [a] | RCI | ADP/O |
|---|---|---|---|
| *Pyruvate + malate* | | | |
| Patient #1 | 076.9 | 4.2 | 3.2 |
| Patient #2 | 114.7 | 5.7 | 2.8 |
| Patient #3 | 45.6 | 1.5 | 3.1 |
| Patient #4 | 97.4 | 2.8 | 2.8 |
| Patient #5 [b] | 32.0 | 2.8 | 2.3 |
| Patient #6 | 60.4 | 3.0 | 3.1 |
| Patient #7 | 68.4 | 3.7 | 3.0 |
| Patient #8 | 40.9 | 1.6 | 3.0 |
| Patient #9 | 81.5 | 4.7 | 2.9 |
| Patient #10 | 52.6 | 3.2 | 2.9 |
| Patient #11 | 19.9 | 1.6 | 2.6 |
| Patient #15 | 111.6 | 4.5 | 2.8 |
| Patient #16 | 61.0 | 2.6 | 2.8 |
| Controls ($n = 6$) | 154 ± 8 | 4.2 ± 0.4 | 3.1 ± 0.2 |
| *Succinate (+rotenone)* | | | |
| Patient #1 | 84.6 | 2.7 | 2.1 |
| Patient #2 | 166.3 | 3.4 | 1.6 |
| Patient #3 | 129.1 | 1.9 | 1.8 |
| Patient #4 | 132.2 | 1.9 | 1.4 |
| Patient #5 [b] | 41.0 | – | – |
| Patient #6 | 91.0 | 2.1 | 1.8 |
| Patient #7 | 94.7 | 1.8 | 1.8 |
| Patient #8 | 55.3 | – | – |
| Patient #9 | 174.6 | 3.5 | 1.7 |
| Patient #10 | 151.4 | 2.9 | 1.9 |
| Patient #11 | 144.8 | 3.1 | 2.0 |
| Patient #15 | 161.5 | 3.7 | 1.7 |
| Patient #16 | 233.3 | 2.4 | 1.9 |
| Controls ($n = 6$) | 175 ± 13 | 3.2 ± 0.2 | 1.8 ± 0.1 |

The respiratory rates and P/O (ADP/O) ratios of the mitochondrial preparations using various substrates were determined polarographically. The reaction mixture consisted of 150 mM sucrose, 25 mM Tris-Cl, 10 mM phosphate (pH 7.4) and 0.7 to 1.0 mg skeletal muscle mitochondria. Final volume: 1.0 ml; temperature: 30° C. Other substrates are as indicated: 5 mM pyruvate, 2.5 mM malate, 5 mM succinate and 2.5 $\mu$M rotenone. The respiratory rate refers to the State 3 rate which was initiated upon the addition of 258–267 $\mu$M ADP. Patient numbers correspond to Table 2.

[a] Values for controls are the mean ± S.E.

[b] Fresh autopsy tissue; RCI: respiratory control.

muscle mitochondria in patients with MELAS (patients #9–11) and MERRF (patients #15 and 16) are summarized in Table 3. With the exception of patient #11, all patients showed mild-severe impairment of NAD-linked substrate oxidation. The rates of succinate oxidation were within the normal range. The cytochrome contents were also comparable to controls, although a mild deficiency in cytochrome *b* ($\approx$ 60% of control) was seen with patient #11. A portion of these studies have been briefly communicated [20,21].

The data clearly demonstrated that all 4 mitochondrial diseases shared one common biochemical abnormality. Electron transfer associated with Complex I was impaired to a varying degree. Those patients with KSS were most severely affected while those with mitochondrial myopathy were much less affected. However, in all cases, the mitochondria were tightly coupled and possessed normal phosphorylating efficiencies with both succinate and NAD-linked substrates.

### 2.3. Design of therapeutic treatment

The general recommendations made to all patients were to avoid extremes of temperature and overexercise. Fever, infection and seizures were promptly treated, adequate hydration was maintained, and drugs known to compromise mitochondrial function (e.g., anticonvulsants such as dilantin and phenobarbital and antibiotics such as tetracycline and chloramphenicol) were avoided.

Menadiol sodium diphosphate (vitamin $K_3$) (20–60 mg/day, in divided doses), ascorbic acid (vitamin C) (1 g/twice per day), $\alpha$-tocopherol (vitamin E) (200 IU/twice per day), (Coenzyme $Q_{10}$ (30–120 mg/day, in divided doses), and methylprednisolone (2–16 mg/every other day, in divided doses) were administered. Vitamins $K_3$ and C have been used as electron transfer mediators to circumvent reduced activity of Complex III. The effective combined use of vitamins $K_3$ and C has been described in the literature [13,22]. Vitamin C, Vitamin E and Coenzyme $Q_{10}$ are used for their antioxidant properties. Effective use of vitamin E [3,9,10] and Coenzyme $Q_{10}$ [3,10,12] have been described. Low dosages of glucocorticoids (such as methylprednisolone) have been shown to result in an increase in muscle strength and decrease in serum lactate [23,24] and to promote the synthesis of a protein inhibitor of phospholipase (lipocortin) [25]. Methylprednisolone has been effectively used in the treatment of mitochondrial diseases [3,10].

All patients were treated and clinically evaluated every 2–4 weeks.

### 2.4. Evaluation of the efficacy of therapeutic intervention. $^{31}$P-NMR, NIRS and clinical / laboratory examination

Patients were carefully monitored for side effects of pharmacotherapy (e.g., hyperglycemia, bleeding disorder, infection, liver or renal dysfunction). During outpatient follow-up visits, patients and families were interviewed by the same neurologist. A complete physical and neurologic examination was performed. Evidence based on physical examination or laboratory study of clinical deterioration was noted, current therapy was reviewed, and if considered suitable, recommendations were made for changes in therapy. Anticonvulsant levels, serum creatine phosphokinase (CPK), lactic acid levels and relevant laboratory studies were routinely performed.

During the course of the study, 5 patients were sent to the University of Pennsylvania to undergo evaluation using $^{31}$P-NMR spectroscopy. All patients underwent a baseline $^{31}$P-NMR spectroscopic evaluation. Pharmacotherapy was

Table 4
Clinical outcome

| Patient # | Clinical syndrome | Time followed (y) | Major medical events [a] | Current clinical status |
|---|---|---|---|---|
| 1. | mitochondrial myopathy | 13 | 0 | functional |
| 2. | mitochondrial myopathy | 10 | 0 | functional |
| 3. | mitochondrial myopathy | 6 | 0 | gainfully employed |
| 4. | mitochondrial myopathy | 4 | 0 | functional |
| 5. | KSS | 3 | 1 | deceased |
| 6. | KSS | 7 | 0 | functional |
| 7. | KSS | 8 | 0 | functional |
| 8. | KSS | 2 | 1 | deceased |
| 9. | MELAS | 8 | 0 | deceased |
| 10. | MELAS | 4 | 1 | deceased |
| 11. | MELAS | 0.5 | 3 | deceased |
| 12. | MELAS | 5 | 1 | deceased |
| 13. | MELAS | 2 | 3 | deceased |
| 14. | MELAS | 1 | 2 | deceased |
| 15. | MERRF | 15 | 0 | deceased |
| 16. | MERRF | 1 | 3 | deceased |

[a] Major medical events are defined as medical events related to the primary diagnosis which resulted in hospitalization of the patient, (e.g., uncontrolled seizures, organ failure, systemic infection).

administered immediately prior to $^{31}$P-NMR evaluation to determine whether any objective improvement occurred with treatment. Ten patients were examined using near-infrared spectroscopy (NIRS) as previously described [26]. Pharmacotherapy was again administered immediately before the test to determine whether improvement could be objectively demonstrated.

The study performed at the University of Pennsylvania to evaluate whether $^{31}$P-NMR spectroscopy did not reveal acute changes associated with the administration of pharmacotherapy. However, the study did document the utility of $^{31}$P-NMR in screening patients with muscle weakness for mitochondrial disease [27]. NIRS was also used as an outcome measure of the efficacy of pharmacotherapy. Although the efficacy study was inconclusive, due to the small number of patients examined, the data did support the usefulness of NIRS as a screening tool for patients with possible mitochondrial myopathy [28].

### 2.5. Clinical outcome

No patient experienced any significant deleterious side effect of the pharmacotherapy. Repeated blood levels of CPK and lactic acid were not predictive of clinical outcome. Table 4 summarizes the clinical outcome data. Cases #5, 8 and 11–16 were pediatric patients. With the exception of patient #15, all patients died fairly soon after diagnosis. They were hospitalized more frequently than the adults and showed severe biochemical abnormalities on biopsy. Cases #5, 8, 11 and 12 died of cardiac failure, patient #10 of renal failure and cases #13, 14 and 16 died due to complications of status epilepticus. The cause of death of cases #9 and 15 was uncertain although a cardiac etiology was presumed. Case #16 was totally noncompliant with her therapy. Among the adult patients, those with mitochondrial myopathy remained functional. Two KSS patients are still alive and functional, although both required cardiac pacemaker placement. Among the adult MELAS patients, case #13 developed repeated episodes of status epilepticus; the last episode was not responsive to anticonvulsant therapy and she died. Cases #9, 10 and 12 survived longer than expected considering the typically poor prognosis associated with their diagnosis. They had few complications related to their disease and were able to lead a comfortable life until they succumbed to their disease. All 3 of these patients died within a 6 month period after the oral form of $K_3$ was taken off the market due to limited public demand. All patients were given oral phytonadione (Vitamin $K_1$) in similar dosages after the oral form of $K_3$ was discontinued.

## 3. Conclusion

The results of this long-term follow-up study are encouraging. The patients appeared to survive longer with less functional disability and medical complications than typically seen in clinical practice. Those patients who had less severe mitochondrial impairment (based on biochemical analysis), who were compliant with their therapy and were treated early in the course of their disease lived longer, were more functional and had fewer medical complications of their disease.

There is no question that therapeutic drug trials are difficult to conduct in patients with mitochondrial diseases. The diseases are rare, heterogenous and short-term, objective measures which are predictive of outcome have not been identified. $^{31}$P-NMR and NIRS deserve further evaluation in larger patient populations as the patient can serve as their own control and the results of intervention are objective.

More complete understanding of the pathophysiology of mitochondrial disease clearly has facilitated the choice of potential pharmacotherapy. Electron transport mediators, free radical scavengers and drugs which block degrading pathways are logical pharmacotherapeutic choices. The development of new compounds, e.g., 21-aminosteroids [29], which are associated with few side effects and are more potent than the traditional glucocorticoids, is encouraging. Potential pharmacologic strategies have recently been reviewed by McIntosh [30].

Although therapeutic drug trials in mitochondrial diseases are inherently difficult to conduct, the long-term benefits are clearly worth the efforts.

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