INTRODUCTION

Defects of the mitochondrial genome are an important cause of genetic disease (1). The clinical features are very varied but in many individuals the defect leads to progressive problems which may result in severe disability and even death. It is against the background of increasing recognition and detection of mitochondrial DNA (mtDNA) disorders that clinicians and scientists must strive to develop effective therapies. In this article we discuss agents that are currently being used in patients (largely unsuccessfully) and possible future therapies.

One of the major difficulties when assessing a potential therapy for mtDNA disorders is the clinical variability of the disease. Thus patients with the same genetic defect may present in a variety of different ways and the disease may take an entirely different course. This means that clinical trials are difficult to perform. In addition, we know relatively little about the natural history of these disorders and collecting enough patients with a similar phenotype in one centre is very difficult. Many drugs used in the treatment of mitochondrial disease therefore only have anecdotal data rather than clinical trials to support their use.

All mitochondrial disorders result from the progressive decline in the ability to supply cellular energy demands in the form of available ATP. The respiratory chain is also a potent source of free radicals, thus a respiratory chain defect can result in ATP deficiency and an excess production of free radicals. Biochemical strategies have sought to increase the production

CURRENT PERSPECTIVES IN THE TREATMENT OF MITOCHONDRIAL DNA DISEASES

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KEY WORDS: Gene therapy, heteroplasmy, mitochondrial DNA, pharmacology, treatment.

FUNCTION NEUROL 2001;16 (SUPPL.): 89-96
of ATP by bypassing the block in electron transfer using artificial electron acceptors, and to enhance residual enzyme activity or minimise the free-radical induced damage that occurs as a result of a defective respiratory chain. When we consider treatment for patients it is important to consider all options and to be realistic about the benefits of respective treatments.

In this short review, we summarise the supportive measures available for patients and a variety of pharmacological agents that have been tried to treat patients with mtDNA defects. Finally we review some of the options for the future.

SUPPORTIVE TREATMENTS

As a mitochondrial dysfunction can present with problems in different systems, it is important that clinicians are aware that other tissues may be involved. Supportive care may significantly improve a patient’s quality of life and help to counteract the progressive, disabling course in some patients. Examples include the appropriate treatment for diabetes and other endocrine disorders, and the management of seizures using anticonvulsants. Ophthalmic splints or corrective surgery may be helpful to patients with progressive external ophthalmoplegia, and surgery can also improve the quality of life of patients with other ocular complications, such as cataracts.

In our experience one problem underestimated in patients with mtDNA defects is dysphagia and in some patients this can be helped by gastrostomy. Cochlear implantation may be of benefit for patients with hearing loss, a common finding in patients with mtDNA defects and in particular the MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes) syndrome (2). Cardiac abnormalities are common and include heart block and other dysrhythmias. These should be identified at an early stage and treated appropriately with either drugs or a pacemaker. Hypertrophic cardiomyopathy can also be an important clinical feature and load-lowering medication should be considered. Successful cardiac transplantation has been reported in patients with Kearns-Sayre syndrome and hypertrophic cardiomyopathy (3).

Although not clinically proven for each drug, there are reasons to avoid treating mitochondrial patients with certain drugs. The anti-convulsant sodium valproate inhibits fatty acid oxidation and oxidative phosphorylation. Chloramphenicol (an inhibitor of mitochondrial protein synthesis) should be avoided. Antiviral agents inhibit the mitochondrial DNA polymerase γ and may cause fatal encephalopathy, in the case of fialuridine (4), or myopathy via mtDNA depletion in the case of the nucleoside analogue azidothymidine (AZT) (5). The penetrance of the mitochondrial A1555G 16S rRNA mutation associated with inherited non-syndromic deafness may be enhanced by exposure to aminoglycoside antibiotics; identification of these individuals and strict avoidance of aminoglycosides may therefore prevent hearing loss (6). Finally, anaesthetic complications may arise in patients with mitochondrial disease, with increased sensitivity to both etomidate and thiopentone documented in patients with Kearns-Sayre syndrome (7).

PHARMACOLOGICAL THERAPY OF MITOCHONDRIAL DISORDERS

Ubiquinone

Ubiquinone (Coenzyme Q10, ubidecarenone) is a lipid-soluble, mobile component of the respiratory chain. It accepts electrons from the flavoprotein moieties of complexes I and II of the respiratory chain and the dehydrogenases of mitochondrial β-oxidation via ETF ubiquinone:oxidoreductase, transferring them sequentially to complex III in the inner membrane by virtue of its ability to become reversibly reduced (to the hydroxyquinone, ubiquinol) and re-oxidised. In addition to its role in electron transfer, ubiquinone is an antioxidant both in lipid membranes and within mitochondria (8).

Whilst patients with ubiquinone deficiency respond very positively to ubiquinone supplementation (9), the administration of ubiquine
(60-150 mg/day) to patients with a variety of other respiratory chain defects may improve the clinical picture. Benefits include an improvement in clinical symptoms (10), CSF lactate levels (11) and parameters of muscle oxidative metabolism as determined by in vivo phosphorus magnetic resonance spectroscopy (31P-MRS) (12). Improvements in cardiac conduction have been noted in Kearns-Sayre syndrome (13), and a correction in the calcium homeostasis of two patients with hypoparathyroidism and mtDNA deletions has also been documented (14). However, such objective improvements in clinical and biochemical parameters are not always apparent in larger clinical trials (15,16). The largest double-blind multicentre trial to date studied 44 patients, 16 of whom appeared to respond to the ubiquinone as shown by a significant decrease in post-exercise lactate levels during the initial trial phase (17). These patients were treated for a further three months (2 mg/kg/day), but no differences were observed from placebo. Nevertheless, many clinicians continue to prescribe ubiquinone and we find that some patients report an improvement in muscle symptoms such as fatigue or cramps on starting treatment.

Idebenone

Idebenone (2,3-dimethoxy-5-methy-6-(10-hydroxy)-decyl-1,4-benzoquinone) is a synthetic analogue of ubiquinone that has been shown to cross the blood-brain barrier and localise in mitochondria (18). Similar to ubiquinone, idebenone may function as an electron carrier in the respiratory chain, promoting a redox bypass of complex I. Consequently, the ability of idebenone to ameliorate functional complex I deficiency and facilitate visual recovery has been studied in a series of patients with Leber's hereditary optic neuropathy (LHON). Twenty-eight LHON patients (each harbouring one of the three primary pathogenic mtDNA mutations) were administered idebenone (90 mg/day) and other vitamin (B2 and C) supplements. The mean interval between the onset of LHON and visual recovery was apparently significantly shorter in the treated than in the untreated group (19). However, spontaneous improvement and recovery of vision may occur in LHON even in the absence of any treatment.

At higher doses (90-270 mg/day), idebenone has been reported to improve brain mitochondrial oxidative metabolism in patients with MELAS (20). In one 36-year-old man, quantitative PET analysis showed an improved cerebral metabolic ratio of oxygen and oxygen extraction in the non-infarcted cerebral cortices without an increase in cerebral blood flow, indicative of a restoration in cerebral mitochondrial oxidative metabolism (20). Two patients given a combination of idebenone and ubiquinone (210 mg/day) demonstrated improvements in both EEG and mental status, with concomitant decrease in CSF protein, lactate and pyruvate suggestive of a therapeutic effect.

Menadione

When administered with ascorbate (vitamin C), the lipid-soluble ubiquinone analogue menadione (vitamin K3) may facilitate the re-oxidation of reduced ubiquinone (ubiquinol), transferring these electrons to cytochrome c, and thus bypass complex III. Oral administration of menadione (10 mg, four times a day) and ascorbate (1g, four times a day) caused functional improvement (31P-MRS) in a patient with a progressive mitochondrial myopathy due to a specific defect in complex III (21). Despite these findings being confirmed by a follow-up study, the pharmacological response in other patients with isolated complex III deficiency (22) and other respiratory chain abnormalities (16) has proved disappointing.

Dichloacetate

The oral hypoglycaemic agent dichloroacetate (DCA) has been shown to enhance residual mitochondrial enzyme activity by inhibiting the protein kinase that reversibly inactivates the pyruvate dehydrogenase complex (PDHC), maintaining PDHC in its unphosphorylated, catalytically active form (23). Consequently, DCA administration (25 mg/kg/day) reduces elevated
serum pyruvate and lactate levels in various metabolic conditions (24), whilst clinical improvement following DCA therapy has been documented in patients with cytochrome c oxidase (COX) deficiency (25), complex I-deficiency (26) and MELAS (27). However, DCA can also have significant adverse effects, including reversible peripheral neuropathy, even with thiamine (100 mg/day) co-medication (28).

Riboflavin

Respiratory chain complexes I and II both contain flavin moieties. Because riboflavin (vitamin B₂) can act as a flavin precursor for both complexes, should the genetic defect in either complex affect flavin synthesis or binding, pharmacological doses of riboflavin may serve to enhance residual enzyme activity. Several studies have reported beneficial effects of riboflavin treatment (100 mg/day) in patients with complex I deficiency (29), although in a separate study, the observed increase in complex I activity failed to correlate with clinical response (30). Such findings highlight not only the obvious limitations of small clinical trials, but also the need for placebo-controlled, double-blinded studies to obtain objective responses in the treatment of mitochondrial disorders.

Creatine

It has been postulated that creatine monohydrate supplements may be of therapeutic benefit as in vivo ³¹P-MRS studies of patients with various mitochondrial abnormalities have shown decreased levels of intramuscular phosphocreatine (31). A double-blind study of seven myopathic patients showed that creatine supplementation increased high-intensity strength and aerobic power without adverse side effects (32), whilst another report documented improved exercise intolerance in a patient with MELAS (33). Short-term creatine monohydrate supplementation (5-10g/day for 2 weeks) to adult patients with other neuromuscular conditions (including inflammatory myopathies, muscular dystrophies and neuropathic disorders) also led to a significant increase in high-intensity strength in the patient group (34).

Carnitine

Carnitine supplements (1-3 g/day) are often administered with ubiquinone to patients with respiratory chain disease to ameliorate the secondary carnitine deficiency, although objective evidence supporting a therapeutic role is limited to a single case in which carnitine was given in combination with other vitamin supplements (35).

Succinate

Succinate can donate electrons directly to complex II, and as such offers a potential treatment for patients with complex I deficiency. Doses of 6g/day were reported to alleviate the stroke-like episodes of a patient with MELAS (36), whilst in a patient with Kearns-Sayre syndrome, administration of succinate together with ubiquinone (300 mg/day) improved respiratory function markedly (37).

POSSIBLE FUTURE THERAPIES FOR mtDNA DISEASE

Such are the shortcomings of the available biochemical and pharmacological therapies that genetic and other experimental strategies to treat patients with mitochondrial disease have started to be explored (reviewed in 38). However, there exist substantial hurdles to developing realistic gene therapies for mtDNA-related diseases. The mitochondrial genome is present in multiple copies in every mitochondrion, and pathogenic mutations are often heteroplasmic, with a mixture of mutant and wild type genomes present within the same cell and tissue (39). As such, the proportion of mutant mtDNA molecules must exceed a critical threshold before a biochemical defect (and hence a clinical phenotype) is apparent. To date, there are no available techniques to manipulate mtDNA gene expression and the targeting of any potential therapeutic molecules to the mitochondrial compartment.
within affected organs will require novel delivery systems to negotiate its highly selective, double-membrane. Based upon the large number of different mtDNA mutations already known to cause disease (>200 have already been characterised), it is likely that any potential therapeutic molecule or strategy will have to be adapted to target specific mitochondrial genetic abnormalities. Here we consider two very different experimental approaches which have shown considerable promise in manipulating the critical determinant in the expression of mtDNA diseases, namely the relative proportions of mutant to wild type mtDNA molecules.

**Agents that manipulate mitochondrial DNA heteroplasmy**

The accumulation over time of specific pathogenic mtDNA mutations suggests that even in post-mitotic cells, mtDNA is replicating (40). The development of molecules that bind to, and specifically inhibit the replication and turnover of mutant mtDNA molecules offers an attractive therapeutic strategy with broad applications. Assuming such agents could be targeted to the mitochondrial matrix, the successful inhibition of mutant genomes over time would theoretically give the wild-type genome a distinct replicative advantage to the extent that the level of heteroplasmy may be altered.

**Antigenomic therapy for disorders of the mitochondrial genome**

The sequence-specific inhibition of mutant mtDNA replication by antigenomic peptide nucleic acids (PNAs) was first demonstrated *in vitro* using an 11-mer PNA designed to span and inhibit specifically at the site of the A8344G MERRF (myoclonus, epilepsy and ragged-red fibre disease) mutation (41). PNAs are uncharged, nuclease-resistant DNA mimics. They bind with high affinity to complementary single-stranded DNA and are readily internalised into various cell types, properties that make them attractive candidate antigen or antisense agents, although any mitochondrial gene therapy using PNAs requires the targeting and import of antigenomic PNAs to mitochondria. Two very different approaches have been used to promote mitochondrial targeting. First, the addition of the targeting sequence of human COX VIII to the PNA appeared to facilitate its uptake into mitochondria within human cells by means of the protein import pathway (42). Second, PNA oligomers have been conjugated to lipophilic phosphonium cations that are actively accumulated in the mitochondrial matrix as a function of the mitochondrial membrane potential (43). More recently, the antigenomic approach has been successfully extended to explore molecules that might selectively bind and inhibit the replication of mtDNA molecules containing pathogenic deletions (44).

**Induced muscle regeneration in mitochondrial DNA disorders**

The diagnosis of two myopathic patients with different pathogenic mtDNA tRNALeu(CUN) gene mutations (40,45) has led to the development of another experimental strategy by which the amount of wild type relative to mutant mtDNA may be increased *in vivo*. Both patients were observed to harbour very high levels of patholog- ical mutation in mature muscle, whilst these mutations were present at very low or undetectable levels in satellite cells (quiescent myogenic precursors) from the same muscles. Using either the anaesthetic bupivacaine (46) or traumatic muscle injury (47) to induce muscle necrosis, the authors hypothesised correctly that the stimulation of muscle regeneration *in vivo* in these patients might restore a normal mitochondrial DNA genotype and biochemical activity. Similar findings (significant increases in the number of COX-positive fibres and proportion of wild type mtDNA) were later observed in one of these patients following concentric resistance exercise training of the affected muscles to enhance the incorporation of satellite cells with low levels of mutation (48). The therapeutic benefit of this strategy remains to be evaluated in larger numbers of patients, and recent studies of aerobic conditioning in patients with various mitochondrial myopathies suggest that the aerobic training might actually induce an
increase in the proportion of mutant mtDNA in some patients (49).

CONCLUDING REMARKS

Disorders of the mitochondrial genome can cause severely debilitating, progressive neurological disease, and there continues to be no effective cure. Here, we have considered many studies in the literature that investigate potential biochemical therapies for mitochondrial disease and document drug efficacy. Whilst administering pharmacological agents such as ubiquinone may be of benefit in individual cases, there is no clear evidence that biochemical therapies improve the symptoms or alter the course of disease for the vast majority of patients with mitochondrial disorders. The recent description of the first mouse model of a pathogenic mtDNA disorder represents a major advance in the field of mitochondrial genetics (50). Such models are critical for testing new drugs and gene therapies that seek to alter the course of disease progression by specifically targeting and influencing the expression of pathogenic mtDNA mutations.

ACKNOWLEDGMENTS

We thank The Wellcome Trust and the Muscular Dystrophy Campaign for their continuing financial support.

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