TREATMENT OF MITOCHONDRIAL DISEASES

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Incurable diseases are not untreatable diseases. Both pharmacological (*e.g.* antiepileptic drugs) and surgical (*e.g.* blepharoplasty) remedies are useful in prolonging and improving the life of mitochondrial patients (palliative therapy[1]).

The most obvious therapeutic approach is to enhance respiratory chain function, thus mitigating both energy crisis (ATP deficit) and oxidative stress (toxic buildup of ROS).

Supplement therapy, more useful than the slangy "cocktail" therapy, has been since the 1980s and still is the predominant treatment in all mitochondrial diseases, suggesting to improve the most bothersome symptoms, fatigue, exercise intolerance, weakness, myalgia, and constipation.

The composition of the "cocktail" includes – in full or in part – the following oral supplements: coenzyme Q_{10} (CoQ_{10}, 200-600 mg daily); alpha-lipoic acid (600 mg daily); riboflavin (100 mg daily); folinic acid (800 mcg daly); L-carnitine (3 g daily); creatine monohydrate (6 g daily); thiamin (900 mg daily); niacin (100 mg dily); pantothenic acid (500 mg daily); vitamin B12 (500 mcg daily); biotin (30 mcg daily); vitamin C (40 mg/daily; vitamin E (400 IU daily).

Disappointing results from "cocktail" therapy have come from a multitude of individual reports and from one relatively recent Cochrane review [2]. The most common supplement, CoQ_{10}, gives beneficial results in occasional patients with primary CoQ_{10} deficiency (due to mutations in enzymes of CoQ_{10} biosynthesis) [3] but not in other mitochondrial diseases. Administration of L-carnitine proves to be useful – even life-saving – in rare children with primary systemic carnitine deficiency (due to mutations in the *SLC22A* gene encoding carnitine transporter [4]), but it has uncertain value in mitochondrial diseases.

All components of the "cocktail" are proven to be safe and well tolerated. Keeping track of the numerous recommended supplements, supplement expenses increased the financial burden to the family. There have been attempts to combine all supplements into one pill.

Generally, hyperoxia (hyperbaric oxygen therapy) is not helpful in mitochondrial diseases and, paradoxically, hypoxia should not be used although beneficial effects were reported experimentally in a transgenic mouse model with Leigh syndrome [5].

A recent review collected thoughtful considerations on "cocktail" therapy of multiple nutritional interventions in primary mitochondrial disorders [6].

Aerobic exercise is a generally accepted beneficial regimen to patients with mitochondrial myopathies, leading to increased mitochondrial biogenesis and improved respiratory chain activities [7].

In a randomized, placebo-controlled trial, idebenone, a CoQ analogue and cofactor for NADPH dehydrogenase, was shown to preserve vision in patients with LHON and discordant vision at baseline and, in the open-labeled follow-up study, preservation of vision persisted for >2 years [8]. Based on these findings, the European Commission has granted marketing authorization for the use of idebenone in patients with LHON.

If it is true that mitochondrial proliferation (*e.g.* the ragged-red fibers in skeletal muscle) is a futile compensatory attempt, we could try to improve on nature's strategy by enhancing mitochondrial biogenesis through the activation of the transcriptional coactivator PGC-1α (peroxisome proliferator-activated receptor γ [PPARγ] coactivator)-1α. The advantage over disease-induced mitochondrial proliferation, which favors mutated mtDNAs, is that pharmacological upregulation of mitochondrial biosynthesis increases the number of *all* mtDNAs, thus allowing wild-type genomes to compensate for mutated ones. Encouraging results have been obtained in four mouse models of COX deficiency: one COX10-deficient animal benefited from treatment with bezafibrate, a PPAR panagonist, [9, 10] as did three transgenic mice (with mutations in *Surf1*, *Sco2*, and *Cox15*) treated with the AMP-dependent kinase (AMPK) agonist AICAR, which phosphorylates PGC-1α.

For mtDNA-related diseases, many of which are devastating and undiagnosable prenatally (at least as far as reliably predicting their clinical expression), the Holy Grail would be to prevent their occurrence altogether via "cytoplasmic transfer." We have long known that this was technically feasible, but recent advances raise the hope that this reproductive option will soon be available to women carrying mtDNA mutations. In this technique, the nucleus of an *in vitro*-fertilized oocyte from a carrier is transferred to an enucleated oocyte from a normal donor: the embryo will have the nDNA of the biological parents but the mtDNA of a normal mitochondrial donor. Experiments in primates using spindle-chromosomal complex transfer (ST) have documented that offspring were healthy and devoid of maternal mtDNA.[11] The same technique has been applied to fertilized and unfertilized but parthenogenically activated[12] human oocytes and has shown that cells develop into normal blastocysts and contain exclusively donor mtDNA. Even more excitingly, only donor mtDNA was detected after stem cell lines from blastocysts were differentiated into neurons, cardiomyocytes, and β-cells.[12] Similar results were obtained in the UK after pronuclear transfer in abnormally fertilized human oocytes developed to the blastocyst stage.[13] Despite some minor concerns, the stage seems set for approval of this technique for therapeutic application both in the UK and in the USA [14].

For nDNA-related mitochondrial disorders, "classical" gene therapy is – of course – an option, although it carries the well-known "baggage" of this approach, including the choice of appropriate viral or non-viral vectors, delivery to the affected tissues, and potential immunological reactions. Nonetheless, adeno-associated virus-mediated gene transfer has proven successful in two animal models, the *Ant1* mutant mouse and in a mouse model of ethylmalonic encephalopathy [15].

A more promising "detoxifying" therapy – despite its inherent risks – appears to be allogeneic hematopoetic stem cell transplantation (AHCT) aimed at restoring sufficient thymidine phosphorylase (TPase) activity in patients with MNGIE to normalize the circulating toxic levels of thymidine and deoxyuridine.[16] As of 2010, 5 of 11 patients with MNGIE who had undergone AHCT were alive, had normal blood TPase activity and virtually undetectable levels of thymidine and deoxyuridine. An international controlled trial showed that 9 of 24 patients were alive, and at last follow-up with a median follow-up of those surviving patients was 1,430 days [17].

Pharmacological strategies modify the course of certain mitochondrial diseases that are caused by metabolic blocks leading to toxic compounds accumulation. N-acetylcusteine and metronidazole treatment can be used to reduce the high levels of hydrogen sulfide that occur in ethylmalonic encephalopathy. Ethylmalonic encephalopathy is a devastating autosomal recessive childhood-onset mitochondrial disease due to mutations in *ETH1*. ETH1 is a mitochondrial sulfur dioxygenase, which detoxifies hydrogen sulfide. Hydrogen sulfide can inhibit the terminal segment of fatty acid beta-oxidation as well as complex IV and directly damages the endothelial lining of small vessels [18]. N-acetylcysteine acts as an intracellular buffer for hydrogen sulfide, and metronidazole is an antibiotic that specifically targets hydrogen sulfide-producing anaerobic bacteria and protozoa in the microbiota – an important source of hydrogen sulfide. Co-administration of N=acetylcysteine and metronidazole has been shown to prolong the lifespan of Ethe-/- mice and mitigate some of the characteristic clinical disease features of ethylmalonic encephalopathy, including chronic diarrhea and diffuse microvasculopathy with acrocyanosis in patients[19]. Treatment also improved alertness and wakefulness and decreased the frequency and duration of epileptic seizures.

Other novel antioxidants that are being tested in clinical trials include EPI-743 (which is hypothesized to increase glutathione levels) and cysteamine bitartrate (also known as RP103, which converts intralysosomal cysteine into cysteine-cysteamine disulfide) for Leigh syndromes [20].

One novel exciting approach is based on a pharmacological treatment of incurable children affected with aninvariably lethal mtDNA depletion due to mutations in the *TK2* gene, which encodes thymidine kinase 2, essential in preserving the mitochondrial nucleoside salvage pathway. In 2014, Drs. Michio Hirano and Caterina Garone reported that molecular bypass therapy with the TK2 products, dCMP and dTMP, prolongs the lifespan of Tk2-deficient ($Tk2^{-/-}$) mice by 2-3 fold [21].

Their studies demonstrated that deoxynucleoside substrate enhancement is a novel therapy, which may ameliorate TK2 deficiency in patients. They have initiated dT+dC in one TK2-deficient child in the US under an emergency IND and have obtained a single-patient IND for this treatment in a second child. In addition, at least 13 TK2-deficient patients in Spain, one in Italy, and two in South American have been treated with dTMP+dCMP, dT+dC, or both sequentially. In all patients, low body mass index and muscle weakness has stabilized or

improved. Dr. Hirano and collaborators are working with a pharmaceutical industry partner to move dT+dC therapy forward into a phase I/II clinical trial of patients with TK2 deficiency.

Although mitochondrial therapy is dismal and arduous, I conclude these notes on an optimistic spirit. As far as treatment of mtDNA-elated diseases, I am confident that employment of mitochondria replacement therapy (MRT) with in vitro fertilization (IVF) will eliminate most incurable mtDNA diseases (MELAS, MERRF, NARP/MILS, and MILS). As far as treatment of nDNA-related diseases, a lot remains to be done but encouraging pharmacological therapies are opening new avenues and other approaches will become available.

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