Review

Current biochemical treatments of mitochondrial respiratory chain disorders

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Abstract

Introduction: The clinical heterogeneity and unpredictable clinical course of mitochondrial respiratory chain (MRC) disorders has hindered the development of effective therapeutic strategies for the treatment of these diseases. Treatment generally involves supportive therapy aimed at enhancing mitochondrial function as well as ameliorating increased reactive oxygen species (ROS) generation which is associated with MRC dysfunction. The purpose of this review is to focus on current biochemical strategies together with those that are being developed to treatment of MRC disorders.

Areas covered: This review focusses on the biochemical strategies that have been developed to augment MRC function (increase electron transport and substrate availability in the MRC), scavenge ROS (antioxidant therapies) and modify mitochondrial biogenesis will be covered. The authors have comprehensively reviewed the literature to provide up to date information on these subjects.

Expert opinion: A consensus needs to be reached on the treatment of MRC disorders, and rather than the use of generic `antioxidant cocktails` case specific therapeutic strategies should be considered for the treatment patients. The inclusion of pharmacotherapies that target MRC function, cellular antioxidant status and mitochondrial biogenesis in the treatment regime of patients may be appropriate to ameliorate the defects in these parameters that contribute to disease pathophysiology.

Article highlights

- Mitochondrial respiratory chain (MRC) disorders are a complex group of diseases that have a heterogeneous clinical presentation and disease course creating formidable challenges for their effective treatment management.
- The majority of therapeutic strategies available for the treatment of MRC disorders focus upon enhancing the activity of the MRC and restoring cellular antioxidant status.
- Pharmaceutical agents that are capable of stimulating mitochondrial biogenesis and in some cases reversing MRC enzyme deficiencies have received a lot of attention over recent years with some of these compounds now entering clinical trials.
- At present there is no consensus or unified approach to the treatment of MRC disorders and therapeutic strategies can vary between countries and specialist centres.

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- Candidate therapies should be determined on a case specific basis. Furthermore, it may be appropriate to include pharmaceutical agents that target MRC function, cellular antioxidant status and mitochondrial biogenesis in the treatment regime of patients in view of the involvement of all of these parameters in disease pathophysiology.

1. Introduction

The mitochondrial respiratory chain (MRC; Figure 1) consists of four enzyme complexes; complex I (NADH: ubiquinone reductase; EC 1.6.5.3); complex II (succinate: ubiquinone reductase; EC 1.3.5.1); complex III (ubiquinol: cytochrome c reductase; EC 1.10.2.2) ; complex IV (cytochrome c oxidase; EC 1.9.3.1) and two mobile electron carriers, coenzyme Q_{10} (CoQ_{10}) and cytochrome c and is located in the inner mitochondrial membrane [1,2]. The MRC together with complex V (ATP synthase; EC 3.6.3.14; Figure 1) synthesizes ATP via the process of oxidative phosphorylation and consequently it is known as the oxidative phosphorylation |(OXPHOS) system [1]. Disorders of oxidative phosphorylation (disorders of the MRC and complex V) exhibit a phenotypic, genetic and biochemical heterogeneity which may reflect the dual genetic origin of the MRC and complex V which are encoded for by both nuclear DNA and mitochondrial DNA (mtDNA) [1]. Other factors such as heteroplasmy, the energy threshold of the tissue and the super-complex formation of the MRC may also contribute to this variability, although other unknown factors may also be involved [1,2]. Furthermore, mutations in genes encoding non-MRC components together with environmental factors may also be important contributory factors to these disorders [2]. In view of the paucity of research and limited clinical trials, there has been limited information available for the development of appropriate therapeutic strategies for the treatment of disorders of oxidative phosphorylation [3]. However, the recent establishment of national and international patient cohorts will provide a readily available patient resource for future clinical trials [4].

Once a diagnosis has been accomplished, most patients with disorders of the MRC and complex V are offered symptomatic treatment [5], and in view of the multisystem presentation of these disorders, a broad range of medical specialties are involved in the management and treatment of these symptoms [6]. The majority of therapeutic strategies available for the treatment of MRC disorders focus upon enhancing the activity of the MRC and/or decreasing the level of oxidative stress (an imbalance between the production of free radicals, e.g. reactive oxygen species (ROS) and the body`s ability to neutralize these species) associated with these conditions [8]. In recent years, there has been

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considerable interest in pharmaceutical treatments aimed at stimulating mitochondrial biogenesis as well as reversing the MRC enzyme defect underlying the disease [9].

This review will focus solely on the biochemical strategies used both currently and that are being developed for the treatment of MRC disorders, although some of these treatments also be applied to patients with complex V defects.

2. Therapeutic strategies that target the mitochondria

The therapeutic strategies that target the mitochondria can be divided into treatments that:

1. Augment MRC function
2. Scavenge ROS (antioxidant therapies)
3. Modify mitochondrial biogenesis.

Details and examples of these strategies are outlined in the following sections.

2.1 Augmentation of MRC function

One of the therapeutic strategies used for the treatment for MRC disorders is to try and increase the rate as well as the efficiency of electron flow in the MRC which would be expected to increase the rate of oxidative phosphorylation with the concomitant production of ATP. The two current strategies used are:

1. Enhancement of the efficiency of electron transport in the MRC.
2. Increasing MRC substrate availability, in some cases by bypassing a defective enzyme complex [10].

Once diagnosed patients with a MRC disorder, are generally treated with a `Mitochondrial Cocktail` which contains antioxidants and various cofactors and vitamins [11]. However, there is no overall consensus on the treatment of patients with these disorders. Commonly used agents which are administered orally as either a monotherapy or as a constituent of the `Mitochondrial Cocktail` include, coenzyme Q_{10} (CoQ_{10}) and its analogue, idebenone and riboflavin (vitamin B2).

2.2 CoQ_{10}

CoQ_{10} serves as an electron carrier in the MRC, transporting electrons derived from complex I and II to complex III and is therefore integral to the process of oxidative phosphorylation [12,13]. CoQ_{10} also serves to transfer electrons liberated from the □-oxidation of fatty acids to complex III of the MRC [14] as well as being involved in DNA replication and repair through its role in pyrimidine synthesis [15]. More recently, CoQ_{10} has been shown to have a role in hydrogen sulfide metabolism where it acts as an electron carrier in the reaction catalysed by sulfide: quinone oxidoreductase [16]. Therefore, in

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addition to an impairment in oxidative phosphorylation a deficiency in CoQ_{10} status may also result in a perturbation of fatty acid b-oxidation, pyrimidine synthesis and hydrogen sulfide oxidation which all may contribute to disease pathophysiology.

Primary CoQ_{10} deficiencies or defects in the CoQ_{10} biosynthetic pathway as the result of mutations in genes encoding for enzymes in the CoQ_{10} biosynthetic pathway and are unique amongst MRC disorders in that they are potentially treatable with high dose oral CoQ_{10} supplementation if diagnosed at an early stage of the disease [13]. Although, not all patients with primary CoQ_{10} deficiency appear to respond to oral CoQ_{10} supplementation, especially those with the neurological presentation of this disorder [13]. The reasons for this are uncertain but may include poor transference across the blood brain barrier (BBB) or irreversible structural damage prior to diagnosis and treatment [13]. Using the analysis of large-scale sequencing data it has been estimated that up to a maximum of 123,789 individuals worldwide may suffer from primary CoQ_{10} deficiency with the majority of these patients as yet to be diagnosed [17].

In view of the possibility that treatment with CoQ_{10} may improve the efficiency of electron transport in patients with MRC disorders, CoQ_{10} supplementation is one of the most consistent therapeutic strategies used in the treatment of these diseases. Although

there is a paucity of high level evidence supporting the use of CoQ_{10} in the treatment of patients with MRC disorders, a number of single and multiple case studies have reported evidence of increased strength, accelerated post exercise recovery and improvement in oxygen consumption in patients following CoQ_{10} supplementation [13,18]. The most consistent finding appears to be a decreased in plasma lactate level following CoQ_{10} supplementation [13,18].

To date, there have only been a limited number of clinical trials that have assessed the efficacy of CoQ_{10} in the treatment of MRC disorders. Unfortunately, the results of these studies have been equivocal with no overall clinical benefit being reported. Although the low bioavailability and dosages of the CoQ_{10} formulations used in these studies together with the short durations of the trials may have contributed to the ambiguity of these results [19,20,21]. Typically, doses of CoQ_{10} in the range, 5-50mg/kg/day are recommended for the treatment of patients with primary CoQ_{10} deficiency and soluble formulations of this agent are suggested rather than tablet forms in view of their higher bioavailability [13,18]. Furthermore, water soluble formulations of ubiquinol, the reduced form of CoQ_{10} have been reported to have better absorption and tissue and mitochondrial uptake than CoQ_{10} and therefore, may prove to be a more effective therapeutic agent than CoQ_{10} [22].

Unfortunately, at present no consensus exists on the appropriate dosages of CoQ_{10} to administer to patients with MRC dysfunction, although a dosage of at least 5mg/kg/day has been recommended [13,18]. Importantly, it has been suggested that patients with MRC disorders who have evidence of a

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secondary CoQ_{10} deficiency (a CoQ_{10} deficiency associated with a mutation in a gene not involved in the CoQ_{10} biosynthetic pathway) may be more responsive to CoQ_{10} therapy and show evidence of clinical improvement following supplementation [23]. Moreover, this illustrates the important of determining the CoQ_{10} status of a patient prior to commencing CoQ_{10} supplementation [18]. It should also be noted that in addition to its ability to improve electron transport in the MRC, the therapeutic efficacy demonstrated by CoQ_{10} in the treatment of MRC disorders may also be the result of its antioxidant capacity [13,18].

In view of the highly lipophilic nature of CoQ_{10} which may limit its uptake into cellular membranes, alternative therapeutic strategies using structural analogues of 4-hydroxybenzoic acid (4-HB), the precursor of the benzoquinone nucleus of CoQ_{10} [24,25,26] have been suggested for the treatment of primary CoQ_{10} deficiencies . Studies in patient fibroblasts harbouring mutations in the CoQ_{10} biosynthetic pathway gene, *CoQ7* and mouse models with a genetic modification in the CoQ_{10} biosynthetic gene, *CoQ9* gene have indicated that the 4-HB analogue, 2,4-dihydroxybenzoate has the potential to restore cellular CoQ_{10} biosynthesis, but this is dependent on the residual level of the CoQ_{10} biosynthetic proteins [24,25]. In addition, the 4-HB and salicyclic analogue, β-resorcyclic acid has been reported to show some therapeutic potential in the treatment of mitochondrial encephalopathy resulting from CoQ_{10} deficiency [26].

2.3 Idebenone

Idebenone is a short chain analogue of CoQ_{10} which is used to treat patients with MRC dysfunction being readily taken up by cells and in contrast to CoQ_{10}, it appears to readily cross the blood brain barrier which is a semipermeable barrier that separates the brain from the blood stream [27]. Idebenone in common with CoQ_{10} acts as an electron carrier in the MRC, however, in contrast to CoQ_{10}, idebenone is thought to transfer electrons directly to complex III, thereby circumventing complex I [13]. Therefore, idebenone is not recommended as a treatment for primary CoQ_{10} deficiencies and has been reported to cause clinical deterioration in patients with this condition and this is supported by the findings of the study by Lopezet al. (2010) which showed that treatment with this analogue was unable to restore the defective mitochondrial energy metabolism in fibroblasts from CoQ_{10} deficient patients [28].

The mechanism by which idebenone is able to transfer electrons directly to MRC complex III, bypassing complex I is thought to result from its ability to act as a substrate for the cytosolic enzyme, NQO1(NAD(P)H dehydrogenase quinone 1) which is able to catalyse the transfer of electrons from NAD(P)H to idebenone [29]. Reduced idebenone then passes its electrons directly to MRC complex III

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enhancing oxidative phosphorylation. Idebenone has been reported to be an effective treatment for the MRC disorder, Leber hereditary optic neuropathy (LHON) which is associated with an MRC complex I deficiency [13]. Idebenone treatment has been reported to prevent the loss and aid the recovery of visual acuity in LHON in addition to preventing the loss of colour vision [30,31]. Idebenone has also been reported to improve cerebral oxidative metabolism in MELAS patients [32]. In addition to acting as an electron carrier in the MRC, idebenone can also act an antioxidant which may also contribute to its clinical efficacy in the treatment of MRC disorders [10].

2.4 Riboflavin

Riboflavin or vitamin B2 acts as a precursor of FMN (Flavin mononucleotide) and FAD (flavin adenine dinucleotide) which are prosthetic groups for MRC complex I and II, respectively [33]. Riboflavin obtained from the diet enters the mitochondria from the cytosol by means of specific transporters and is then converted into either FMN and FAD [33]. Therefore, riboflavin transporters are essential for the maintenance of mitochondrial riboflavin homeostasis which if impaired can result in MRC dysfunction with a consequent impairment of oxidative phosphorylation [33]. This is illustrated in cases of Brown Vialetto Van Laere disease which is associated with riboflavin transporter detects and a concomitant impairment in the activities MRC complex I and II in patient fibroblasts (skin cells) [34]. Patients with this riboflavin transporter defect have been reported to show clinical improvement following high dose riboflavin therapy [35]. Furthermore, patients with mutations in the ACAD9, the FADH2 dependent MRC complex I assembly factor have also been reported to show clinical improvement following treatment with riboflavin [36]. Commensurate with this clinical improve, an increased in MRC complex I activity was also reported in fibroblasts from patients with this condition following supplementation with riboflavin [37]. It has been suggested that the increase in the intra-mitochondrial FAD concentration following riboflavin supplementation may improve the folding capacity of mutant flavoprotein assembly factors [38].

The biochemical efficacy of riboflavin therapy for MRC disorders not associated with riboflavin transporter defects or mutations in riboflavin dependent proteins is thought to result from the ability of riboflavin to maximize residual MRC complex I and II activities or enhance the assembly of the defective enzyme complexes [39].

3. Antioxidant therapies

Mitochondria are major sites of ROS generation within the cell and under normal circumstances, the level of cellular ROS is tightly controlled by antioxidants and antioxidant enzyme systems having a physiologically important role in cell signaling [13,40]. However, in cases of MRC dysfunction, electron leakage from the chain can cause increased ROS production which can eventually overwhelm cellular

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antioxidant defenses resulting in oxidative stress [13,41]. Under these conditions, oxidative damage can occur to the MRC enzyme complexes, mtDNA and mitochondrial membrane phospholipids, such as cardiolipin [13,41]. This can result in further damage to the MRC, increased ROS generation and extramitochondrial oxidative damage which can contribute to cellular and consequently organ morbidity [18,41,42]. In view of the detrimental effects of oxidative stress, therapeutic strategies targeted at replenishing cellular antioxidant status have shown some benefit in the treatment of patients with MRC disorders [12,13, 41]. Apart from CoQ_{10} and idebenone which were discussed in the previous sections of this chapter, other antioxidants can be administered to patients with MRC disorders and some notable ones will discussed in the subsequent two sections.

3.1 EPI-743

The tripeptide, reduced glutathione (GSH) serves a major cellular antioxidant being present in high concentrations with the mitochondrion [43]. Although GSH can serve as an antioxidant in its own right, it also serves as a cofactor for the enzyme, glutathione peroxidase which reduces lipid peroxides and hydrogen peroxide to water [44]. Evidence of decreased GSH levels have been reported in both in skeletal muscle [43] and white blood cells [45] of patients with MRC disorders. For this reason, there has been a lot of interest in developing therapeutic strategies to replenish cellular GSH status in patients with MRC disorders. Amongst the compounds which have been studied, the synthetic quinone, EPI-743 has shown particular promise [45]. Although the precise mechanism of action of EPI-743 has yet to be fully elucidated, it is thought to replenish cellular GSH status by its ability to transfer electrons from NOQ1 [29] to the enzyme, glutathione reductase [46]. In children with the encephalopathic mitochondrial disease, Leigh syndrome, EPI-743 treatment was reported to cause a reversal of disease progression [47]. Interestingly, clinical improvement was accompanied by a significant increase in the GSH status of patient lymphocytes following EPI-743 treatment [47].

3.2 Modify mitochondrial biogenesis

The Stimulation of mitochondrial biogenesis has received a lot of attention over recent years on the premise that as MRC disorders are associated with an impairment in oxidative phosphorylation, the ability to increase the mitochondrial content of the cell may therefore compensate for the deficit in cellular energy generation. Evidence of increased mitochondrial biogenesis has been reported in patients with MRC disorders and is thought to represent an adaptive response to the perturbation in cellular oxidative phosphorylation [9].

Mitochondrial biogenesis is under strict regulatory control with the transcriptional co-activator peroxisome proliferator activated receptor γ co-activator-1α (PGC-1α) coordinating the activation

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of a host of transcription factors. These include the nuclear respiratory factors (NRF1+2) and the hormone receptors, PPARs (peroxisome proliferator activated receptor; nuclear hormone receptors) which control the expression of a number of genes (nuclear and mtDNA) encoding components of the MRC as well as proteins involved in their assembly [48.49] (Figure 2). Consequently, the up-regulation of PGC-1α signaling could promote an increase in mitochondrial biogenesis and consequently, the number of mitochondria within the cell. On a cautionary note, PGC-1α stimulates the biogenesis of all mitochondria within the cell whether functional or dysfunctional and therefore, it is uncertain as yet whether this therapeutic approach will ultimately prove beneficial to all patients with MRC disorders, and this requires further study.

PGC-1α is activated by either deacetylation (carried out by sirtuin 1; Sirt1, Figure 2), phosphorylation (carried out by AMP-activated protein kinase; AMPK, Figure 1) or by PPAR γ which up-regulates its gene expression (Figure 2) [51,52]. Pharmaceutical strategies which stimulate these different activation mechanisms have the potential to promote mitochondrial biogenesis and improve residual MRC function.

3.3 PPAR γ activators

Bezafibrate which is commonly used in the treatment of lipid disorders is a pan-agonist activating all three of PPAR isoforms (α, β/δ and γ)[52]. However, only activation of the PPAR γ isoform has only been associated with increased PGC-1α gene expression in skeletal muscle [51] (Figure 2). Studies in patient fibroblasts [53] and animal models of MRC disease [54] have reported evidence of an improvement in MRC function and increased mitochondrial biogenesis following bezafibrate treatment. However, in some mouse models of MRC complex IV deficiency, bezafibrate was not found to be effective in ameliorating MRC dysfunction [55,56].

In vitro studies have indicated that the decanoic acid component of MCT ketogenic diet has the potential to induce mitochondrial biogenesis and also increase MRC function via its ability to act as a PPAR-γ agonist [57]. Decanoic acid treatment of fibroblasts from MRC complex I deficient patients was found to significantly increase the level of mitochondrial enrichment of the cell, although no improvement in complex I activity was noted [58]. Studies in animal models [59,60] and case studies of MRC disease patients [61,62] have reported beneficial effects such as improvement in MRC function, increased cellular antioxidant status and amelioration of clinical symptoms following treatment with the various forms of the ketogenic diet.

3.4 SIRT1 activators

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Another promising therapeutic strategy to activate PGC-1α is by increasing the cellular pool of NAD^+ and thereby increasing the activity of the $NAD^{+,}$ dependent deacetylase, SIRT1 [63]. SIRT1 then deacetylates and consequently activates PGC-1α initiating mitochondrial biogenesis (Fig 2) [50]. Strategies to increase the cellular pool of NAD^+ include supplementing with nicotinamide riboside [63] or pharmaceutical inhibition of the NAD^+ consuming enzyme, poly ADP polymerase 1 (PARP) [64]. Nicotinamide riboside treatment has been reported to boost cellular levels of NAD^+, induce mitochondrial biogenesis and ameliorate clinical symptoms in mouse models of mitochondrial disease [9,11,49]. Although, nicotinamide riboside treatment was not found to produce any beneficial therapeutic effect in a mouse model of MRC complex III deficiency, possibly as a consequence of the underlying starvation-like metabolic state of the mice [65].

3.5 AMPK activation

The AMP (adenosine monophosphate) analogue, 5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR) has been reported to promote mitochondrial biogenesis by its ability to activate AMPK activity and stimulate the PGC-1α signaling cascade [66]. Studies in MRC complex I deficient patient fibroblasts have reported an increase in mitochondrial biogenesis and ATP production following treatment with AICAR [67]. In addition, AICAR treatment was also found to reduce the level of cellular oxidative stress in the fibroblasts [67]. Furthermore, AICAR treatment in mouse models of MRC complex IV deficiency was found to increase in complex IV activity in addition to improving exercise endurance [55]. However, due to its poor availability, short half and association with increased blood lactate and urate levels, the suitability of AICAR for use in patients with MRC disorders is questionable [9].

4. Other treatments

In mtDNA depletion syndrome resulting from a deficiency in the enzyme, thymidine kinase 2 (TK2), supplementation with deoxynucleotides, deoxycytidine monophosphate and deoxythymidine monophosphate has shown some promise in the treatment of a mouse model of this disorder with a delay in disease onset and increased life span being reported [68]. Furthermore, a recent open-label study has indicated the beneficial therapeutic effects of deoxynucleoside monophosphate and deoxynucleoside therapies in the treatment of TK2 deficiency in both children and adult patients [69]. The antiobiotic compound, rapamycin has emerged as potential therapeutic candidate for the treatment of MRC disorders being reported to ameliorate the clinical and biochemical phenotypes of cell and mouse models of mitochondrial disease [70,71,72,73,74,75,76]. Cells and animals harbouring a complex I deficiency appear to particularly benefit from rapamycin treatment possibly as a

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consequence of the antiobiotic`s ability to increase cellular energy status to some degree [70,71,72,73]. Rapamycin is able to inhibit the kinase function of the cytosolic serine/threonine kinase, mTOR1 which is a major cellular metabolic regulator, controlling the transition between anabolic and catabolic states as well as stimulating the *de nova* synthesis of nucleotides, lipids and proteins and inhibiting autophagy [77]. However, the involvement of mTOR1 in mitochondrial disease and mechanism of action of rapamycin in the treatment of these disorders has yet to be completely elucidated [76]. Interestingly, a recent study by Barriocanal-Casado et al (2019) [78] which reported no therapeutic benefit following the treatment of a mouse model of CoQ_{10} deficiency with rapamycin has indicated that rapamycin therapy may not be a suitable treatment for all mitochondrial disease patients and that candidate therapies should be determined on a case specific basis.

5. Conclusion

The pathophysiology of mitochondrial disease is notoriously complex and unpredictable creating formidable challenges for its effective treatment management. Following symptomatic treatment, therapeutic strategies generally focus upon enhancing MRC function and cellular energy generation as well as ameliorating oxidative stress. However, no consensus or unified approach exists at present to utilize these strategies effectively in the treatment of this disparate disease. This may in part be attributable to the paucity of data available from clinical studies assessing therapeutic agents together with the heterogeneity of the MRC disease patient population. Together with the use of `Mitochondrial Cocktails` containing MRC cofactors and antioxidants, the addition of agents to enhance mitochondrial biogenesis may also be beneficial to patients with MRC disorders, although these strategies should be investigated in cell and animals of the disease before being translated to human studies.

6. Expert opinion

Although the therapeutic strategies to augment MRC function discussed in this review are currently used as part of the treatment regime of patients, other agents, especially those that have been reported to elicit mitochondrial biogenesis are only now beginning to be translated from animal studies into clinical trials in human subjects. Therefore, it may be sometime before patients are afford the potential beneficial effects of these pharmacotherapies. In view of the multiplicity of factors that may contribute to the pathophysiology of MRC disorders, therapeutic strategies encompassing agents that target MRC function, cellular antioxidant status and mitochondrial biogenesis may be more appropriate than the `Mitochondrial Cocktails` that are used to treat patients at present [11].

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Furthermore, in view of the heterogeneous nature of MRC disorders, the instigation of specific patient based candidate therapies may be judicious for the treatment of these diseases [78].

Currently, there are two major challenges that impact on the treatment of MRC patients. Firstly, the lack of a consensus or a unified approach to the treatment of MRC disorders. Although the establishment of national and international patient cohorts will provide a resource for the assessment of therapeutic strategies, at present, there is no international body that offers a platform for clinicians and scientists to debate the benefits and disadvantages of therapeutic regimes [4]. Secondly, the lack of reliable validated biomarkers or surrogates available for evaluating mitochondrial function in patients has hampered the ability to assess the clinical utility of candidate therapies [79]

At present, there is some uncertainties as to what will constitute the appropriate therapeutic strategies for the treatment of MRC disorders and regimes encompassing a multiplicity of pharmacotherapies to target MRC function, cellular antioxidant status and mitochondrial biogenesis may be the most appropriate. Unfortunately, the use of combined pharmacotherapies raises the possibility of adverse drug interactions resulting in cellular toxicity and exacerbating patient morbidity [80]. Therefore, the use of single compounds that have the potential to target both MRC dysfunction as well as oxidative stress may be appropriate candidate therapies to treat patients with MRC disorders. Phytochemicals such as curcumin, resveratrol and quercetin appear to fulfill these criteria as demonstrated by *in vitro* cell culture and *in vivo* animals studies in addition to their ability to cross the BBB and enter the CNS [81,82,83]. Interestingly, a phase III clinical trial (Phase III clinical trial involves testing a drug on patients to assess its clinical efficacy and safety) has recently been completed in Thailand in which 70 patients with LHON were treated with curcumin **(**www.clinicaltrials.gov, NCT00528151)**,** although the results of this study have yet to be reported. The author is unaware of any clinical studies that have assessed the efficacy of resveratrol treatment in primary MRC mitochondrial disease, although an open-label clinical trial (a clinical trial in which patients and investigators know which agent the patients are receiving) in Friedrich`s ataxia patients has reported improvement in neurological function, evidence of decreased oxidative, with no serious adverse side effects being recorded [83]. Although quercetin supplementation has been reported to reduce markers of oxidative stress and inflammation in healthy volunteers, no human studies have so far evaluated the effect of this flavonoid on the treatment of MRC dysfunction [85]. Although the reported low bioavailability of quercetin has limited its therapeutic potential, the construction of various quercetin-involved nanoparticles may be commensurate with its use as a future candidate therapy for the treatment of MRC disorders [86]. Indeed, the pharmacological manipulation of both resveratrol and curcumin may also be a consideration to enhance their bioavailability and therapeutic

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potential. However, evidence of cellular toxicity would be required before translation into human studies with these modified phytochemicals.

It is difficult to speculate how the treatment of MRC disorders will evolve in the future, although on a biochemical level, patient specific candidate therapies may have gained momentum and treatment regimes may encompass a variety of agents to target the specific aberrant parameter, be it energy metabolism or oxidative stress. However, together with biochemical agents, gene therapy and stem cell techniques may also be required to elicit some beneficial effects to patients. Importantly, until reliable biomarkers of MRC disorders become available, the potential therapeutic benefit of treatment strategies for these diseases may be underestimated.

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References

[1] Saraste M. Oxidative phosphorylation at the fin de siècle. Science. 1999; 283; 1488-93.

[2] Niyazov DM Kahler SG,. Frye RE. Primary mitochondrial disease and secondary mitochondrial dysfunction: Importance of distinction for diagnosis and treatment. Mol Syndromol. 2016;7: 122–137.

[3] Pfeffer G, Majamaa K, Turnbull DM, et al. Treatment for mitochondrial disorders. Cochrane Database Syst Rev. 2012; 4:cd004426.

[4] Nesbitt V, Pitcheathly R, Cockell S, et al. The Medical Research Council neuromuscular centre for translational research mitochondrial disease patient cohort study UK. J Neurol Neurosurg Psychiatry. 2013; 84: e2.

[5] Rahman S, Hanna M G. Diagnosis and therapy in neuromuscular disorders: diagnosis and new treatments in mitochondrial disease. J. Neurol. Neurosurg. Psychiatry, 2009; 80: 943-53.

[6] Pitceathy RDS, Rahman S, Hanna MG. Single deletions in mitochondrial DNA-Molecular mechanisms and disease phenotypes in clinical practice. Neuromuscul Disord, 2012 ; 22(7) ; 577-87.

[7] Karaa A, Kriger J, Grier J, et al. Mitochondrial disease patients` perception of dietary supplement use. Molec Genet Metab. 2016; 119;100-108.

[8] Kanabus M, Heales SJ, Rahman S. Development of pharmacological strategies for mitochondrial disorders. Br J Pharmacol, 2014; 171(8): 1798-1817.

[9] Poole OV, Hanna MG, Pitceathly RD. Mitochondrial disorders: disease mechanisms ,and therapeutic approaches. Discov Med. 2015;,20(111): 325-31

[10] Rai PK, Russell OM, Lighttowler RN, et al. Potential compounds for the treatment of mitochondrial disease. British Medical Bulletin;2015: 116,5-18.

[11] Nightingale H, Pfeffer G, Bargiela D, et al. Emerging therapies for mitochondrial disorders. Brain. 2016; 139:1633-48.

[12] Kerr DS. Treatment for mitochondria disorders: a review of clinical trials over the past decade. Mol Genet Metab.2010; 99(3):246-255.

[13] Hargreaves IP. Coenzyme Q10 as a therapy for mitochondrial disease. Int J Biochem Cell Biology. 2014; 49: 105-111.

[14] Garcia-Corzo L, Luna-Sanchez M, *Doerrier k, et al.* Dysfunctional Coq9 protein causes predominant encephalomyopathy associated with CoQ deficiency. Hum. Mol. Genet. 2013; 22 (6): 1233-48

Information Classification: General

[15] Lopez-Martin JM, Salviati L, Trevisson E, et al. Missense mutation of the CoQ2 gene causes defects of bioenergetics anddefects of bioenergetics and de novo pyrimidine synthesis. Hum Mol Genet. 2007;16:1091–7.

[16] Luna-Sanchez M, Hidalgo-Gutierrez A, Hildebrandt TM, et al. CoQ deficiency causes disruption of mitochondrial sulfide oxidation, a new pathomechanism associated with this syndrome. EMBO Mol Med. 2016; 9(1): 78-95.

[17] Hughes BG, Harrison PM, Hekimi S. Estimating the occurrence of primary ubiquinone deficiency by analysis of large-scale sequencing data. Scientific Reports.2017; 7: 17744.

18] Neergheen V, Chalasani A, Wainwright L, et al. Coenzyme Q_{10} in the treatment of mitochondrial disease. J Inborn Errors of Metab Screening. 2017; 5: 1-8.

[19] Bresolin N, Doriguzzi C, Ponzetto C, et al. Ubidecarenone in the treatment of mitochondrial myopathies: a multi-centre double-blind trial. J Neurol Sci. 1990; 100(1-2): 70-78.

[20] Chen RS, Huang CC, Chu NS. Coenzyme Q_{10} treatment in mitochondrial encephalomyopathies. Short-term double-blind, crossover study. Eur Neurol, 1997;37(4): 212-18.

[21] Glover EL, Martin J, Maher A, et al. A randomized trial of coenzyme Q_{10} in mitochondrial disorders. Muscle Nerve. 2010; 42(5):739-48.

[22] Garcia-Corzo, L, Luna-Sanchez, M, Doerrier C,et al. Ubiquinol-10 ameliorates mitochondrial encephalopathy associated with CoQ10 deficiency. Biochim Biophys Acta. 2014;1842(7):893–901.

[23] Sacconi S, Trevisson E, Salviati L, et al. Coenzyme Q_{10} is frequently reduced in muscle of patients with mitochondrial myopathy. Neuromuscular Disorders, 2010; 20: 44-48.

[24] Luna-Sanchez M, Diaz-Casado E. Barca E, et al. The clinical heterogeneity of coenzyme Q10 deficiency results from genotypic differences in the CoQ9 gene. EMBO Mol Med. 2015; 7:670-87.

[25] Wang Y, Smith C. Parboosingh JS. Pathogenicity of two COQ7 mutations and responses to 2,4-dihydroxybenzoate bypass treatment. J Cell Mol Med. 2017; 21: 2329-43.

[26] Hidalgo-Gutierrez A, Barriocanal-Casado E, Bakkali M, et al. b-RA reduces DMQ/CoQ ratio and rescues the encephalopathic phenotype in $CoQ9^{R239X}$ mice. EMBO Mol Med. 2019;11: 1-18

[27] Kerr DS. Review of clinical trials for mitochondrial disorders:1997-2012. *Neurotherapeutics,*2013;10:307-319

[28] Lopez LC, Quinzii CM, Area E. Treatment of CoQ10 deficient fibroblasts with ubiquinone, CoQ analogs, and vitamin C: time- and compound-dependent effects. PLoS ONE, 2010;5:e11897.

Information Classification: General

[29] Haefeli RH, Erb M, Gemperti AC, et al. NQO1-dependent redox cycling of idebenone: effects on cellular redox potential and energy levels. *Plos ONE*, 2011; 6:e17963.

[30] Klopstock T, Yu-Wai-Man P, Dimitriadis K, et al. A randomized placebo-controlled trial of idebenone in Leber`s hereditary optic neuropathy. Brain, 2011; 134: 2677-86.

[31] Rudolph G, Dimitriadis K, Buchner B, et al. Effects of idebenone on colour vision in patients with Lebers hereditary optic neuropathy. J Neuro-Ophthalmol. 2013; 33:30-36.

[32] Ikejiri Y, Mori E, Ishii K, et al. Idebenone improves cerebral mitochondrial oxidative metabolism in a patient with MELAS. Neurology. 1996;47: 583-85.

[33] Udhayabanu U, Manole A, Rajeshwari M, et al. Riboflavin Responsive Mitochondrial Dysfunction in Neurodegenerative Diseases. J Clin Med. 2017; 6(5): 52; doi:10.3390/jcm6050052

[34] Manole A, Jaunmuktane Z, Hargreaves I, et al. Clinical, pathological and functional characterization of riboflavin-responsive neuropathy. Brain. 205; 140(11):2820-37.

[35] Foley AR, Menezes MP, Pandraud A, et al. Treatable childhood neurononopathy caused by mutations in riboflavin transporter RFVT2. Brain. 2014;137(Pt 1):44-56.

[36] Gerards M, van den Bosch BJ, Danhauser K *et al*. Riboflavin-responsive oxidative phosphorylation complex I deficiency caused by defective ACAD9: a new function for an old gene. Brain, 2011; 134: 210-19.

[37] Haack TB, Danhauser K, Haberberger B, et al. Exome sequencing identifies ACAD9 mutations as a cause of complex I deficiency. Nat Genet. 2010, 42(12): 1131-34.

[38] Gerards M, Van den Bosch BJC, Danhauser K, et al. Riboflavin-responsive oxidative phosphorylation complex I deficiency caused by defective *ACAD9*: new function for an old gene. Brai*.,* 2011; 134(1):210–19.

[39] Grad L.I, Lemire BD,. Riboflavin enhances the assembly of mitochondrial cytochrome c oxidase in *C. elegans* NADH-ubiquinone oxidoreductase mutants. Biochim. Biophys. Acta Bioenerg. 2006; 1757: 115–22.

[40] Schieber M, Chandel NS. ROS Function in Redox Signalling and Oxidative Stress. Current Biology. 2014, 24(10): 453-462.

[41] Wallace DC, Fan W, Procaccio V, Mitochondrial energetics and therapeutics. Annu Rev Pathol. 2010; 5: 297-348.

Information Classification: General

[42] Hargreaves IP, Duncan AJ, Wu L. Inhibition of mitochondrial complex IV leads to a secondary loss of complex II-III activity:implications for the pathogenesis and treatment of mitochondrial encephalomyopathies. Mitochondrion. 2007; 7: 284-87.

[43] Hargreaves IP, Sheena Y, Land JM, et al. Glutathione deficiency in patients with mitochondrial disease: Implications for pathogenesis and treatment. J Inherit Metab Dis, 2005. 28:81-8.

[44] Stepien KM, Heaton R, Rankin S, et al. Evidence of Oxidative Stress and Secondary Mitochondrial Dysfunction in Metabolic and Non-Metabolic Disorders. J Clin Med. 2017; 6(7): pii: E71.

[45] Pastore A, Petrillo S, Tozzi G, et al. Glutathione: a redox signature in monitoring EPI-743 therapy in children with mitochondrial encephalomyopathies. Mol Genet Metab. 2013; 109(2): 208-14.

[46] Martinelli D, Catteruccia M, Piemonte F, et al. EPI-743 reverses the progression of the paediatric mitochondrial disease. Mol Genet Metab. 2012; 107: 383-388.

[47] Enns GM, Cowan TM. Glutathione as a Redox Biomarker in Mitochondrial Disease—Implications for Therapy. J Clin Med. 2017; 6(5):50.

[48] Schon EA, Dimauro S, Hirano M, et al. Therapeutic prospects for mitochondrial disease. Trends in Molecular Medicine. 2010;16: 268-76.

[49] Viscomi C. Toward a therapy for mitochondrial disease. Biochem Soc Trans. 2016 ; 4(5) : 1483-90.

[50] Puigserver P, Spiegelman BM. Peroxisome proliferator-activated receptor-□□□PGC-1□□□ transcriptional coactivator and metabolic regulator. Endocr Rev. 2003; 24:78-90.

[51] Hondares E, Pineda-Torra I, Iglesias R, et al. PPAR □ but not PPAR □ activates PGC-1□ gene transcription in muscle. Biochem Biophys Res Commun. 2007; 354: 1021-27.

[52] Kliewer SA, Lehman JM, Wilson TM. Orphan nuclear receptors:shifting endocrinology into reverse. Science. 1990; 284: 757-60.

[53] Bastin J, Aubey F, Rotig A, et al. Activation of peroxisomal proliferators activated receptor pathway stimulated the mitochondrial respiratory chain and can ciorrect deficiencies in patients lacking its components. J Clin Endocr Metab. 2008 ; 93 : 1433-41.

[54] Noe N, L. Dillon, Lellek V, et al. Bezafibrate improves mitochondrial function in the CNS of a mouse model of mitochondrial encephalopathy. Mitochondrion. 2013; 13: 417-26

[55] Viscomi C, Bottani E, Civiletto G, et al. In vivo correction of COX deficiency by activation of the AMPK/PGC-1a axis. Cell Metab, 2011; 14: 80-90.

Information Classification: General

[56] Yatsuga S, Suomalainen A. Effect of bezafibrate treatment on late-onset mitochondrial myopathy in mice. Hum Mol Genet. 2012; 21:526-35.

[57] Hughes SD, Kanabus M, Anderson et al. The ketogenic diet component decanoic acid increases mitochondrial citrate synthase and complex I activity in neuronal cells. J Neurochem. 2014; 129: 429-33.

[58] Kanabus M, Fassone E, Hughes SD, et al. The pleiotropic effects of decanoic acid treatment on mitochondrial function in fibroblasts from patients with complex I deficient Leigh syndrome. J Inherit Metab Dis. 2016;39(3): 415-26.

[59] Sullivan PG, Rippy NA, Dorenbos K, The ketogenic diet increases mitochondrial uncoupling protein levels and activity. Ann Neurol, 2004; 55: 576–80.

[60] Jarrett SG, Milder JB, Liang LP, et al. The ketogenic diet increases mitochondrial glutathione levels. J Neurochem. 2008 ;106 :1044–51.

[61] Kang HC, Kim HD, Lee YM, et al. Landau-Kleffner syndrome with mitochondrial respiratory chain-complex I deficiency. Pediatr Neurol, 2006; 35:158–61.

[62] Seo JH, Lee YM, Lee JS, et al. A case of Ohtahara syndrome with mitochondrial respiratory chain complex I deficiency. Brain Dev. 2010 ;32 : 253–57.

[63] Khan NA, Auranen M, Paetau I, et al. Effective treatment of mitochondrial myopathy by nicotinamide riboside, a vitamin B3. EMBO Mol Med. 2014; 6: 721-31.

[64] Cerutti R, Pirinen E, Lamperti C, et al. NAD(+)-dependent activation of Sirt1 corrects the phenotype in a mouse model of mitochondrial disease. Cell Metab, 2014 ; 19 : 1042-49.

[65] Purhonen J, Rajendran J, Tegelberg g, et al. NAD+ repletion produces no therapeutic effect in mice with respiratory chain complex III deficiency and chronic energy deprivation. FASEB J. 2018 ; https://doi.org/10.1096/fj.201800090R

[66] AndreuX PA, Houtkooper RH, Auwerx J. Pharmacological approaches to restore mitochondrial function. Nat Rev Drug Discov. 2013;12(6):465-83.

[67] Golubitzky A, Dan P, Weissman S, et al. Screening for active small molecules in mitochondrial complex I deficient patient`s fibroblasts, reveals AICAR as the most beneficial compound. PloS.2011;6: e26883.

[68] Lopez-Gomez C, Levy RJ, Sanchez-Quintero MJ, et al. Deoxycytidine and deoxythymidine treatment for thymidine kinase 2 deficiency. Ann Neurol, 2017; 81: 641-52.

[69] Dominguez-Gonalez C, Madruga-Garrid M. Mavillard F, et al. Deoxynucleoside therapy for thymidine kinase 2 (TK2) deficient myopathy. Ann Neurol. 2019; doi: 10.1002/ana.25506.

[70] Johnson SC, Yanos ME, Bitto A, et al. Dose-dependent effects of mTOR inhibition on weight and mitocondrial disease in mice. Front Genet. 2015; 6: 247.

Information Classification: General

[71] Johnson SC, Yanos ME, Kayser EB, et al. mTOR inhibition alleviates mitochondrial disease in a mouse model of Leigh síndrome. Science. 2013; 342: 1524-28.

[72] Zheng X, Boyer L, Jin M , et al. Alleviation of neuronal energy deficiency by Mtor inhibition as a treatment for mitocondrial –related neurodegeneration. Elife. 2016; 5: E13378.

[73] Felici R, Buonvicino D, Muzzo M, et al. Post onset, oral rapamycin treatment delays development of mitochondrial encephalopathy only at suparmaximal doses. Neuropharmacology. 2017; 117: 74-84.

[74] Khan NA, Nikkanen J, Yatsuga S, et al. Mtorc1 regulates mitochondrial integrated stress response and mitochondrial myopathy progression. Cell Metab.2017; 26: 419-28.

[75] Siegmund SE, Yang H, et al. Low-dose rapamycin extends lifespan in mouse model of mtDNA depletion síndrome. Hum Mol Genet 2017. 26. 4588-4605.

[76] Civiletto G, Dogan SA, Cerutti R, et al. Rapamycin rescues mitochondrial myopathy via coordinated activation of autophagy and lysosomal biogénesis. EMBO Mol Med. 2018; 10: e8799.

[77] Saxton RA, Sabatini DM. mTOR signalling in growth, metabolism and disease. Cell. 2017; 169: 361-71.

[78] Barriocanal-Cesado E, Hidalgo-Gutierrez A, Raimundo N, et al. Rapamycin administration is not a valid therapeutic strategy for every case of mitochondrial disease. EBioMedicine. 2019; 42: 51123.

[79] Hargreaves IP. Biochemical Assessment and Monitoring of Mitochondrial Disease. J Clin Med. 2018; 7(4): 66

[80] Abubakar AR, Simbak NB, Haque M. Adverse drug reactions: predisposing factors, modern classifications and causality assessment. Res J Pharm Tech. 2014;7(9):1091–8.

[81]. Paulson OB. Blood brain barrier, brain metabolism and cerebral blood flow. European Neuropsychopharmacology, 2002; 12(6): 495-501.

[82]. Mishra S, Palanivelu k. The effect of curcumin (turmeric) on Alzheimer`s disease: An overview. Ann Indian Acad Neurol. 2008; 11(1): 13-29.

[83] Mehrota A, Sandhir R. Quercetin supplemrntation is effective in improving mitochondrial dysfunctions induced by 3-nitropropionic acid: Implications in Huntington`s disease. Biochimica et Biophysica Acta. 2013; 1832:421-30.

[84] Yiu EM, Tai G, Peverill RE, et al. An open-label trial in Friedreich ataxia suggests clinical benefit with high-dose resveratrol, without effect on frataxin levels. J Neurol. 2015; 262(5) : 1344-53.

Information Classification: General

[85] Gholamreza A, Ghiasvand R, Feizi A, et al. The effect of quercetin supplementation on selected markers of inflammation and oxidative stress. J Res Med Sci. 2012; 17(7): 637-641.
[86] Amanzadeh E, Esmaeilj A, Nourbakshnia M. Application of quercetin in neurological disorders: from nutrition to nanomedicine. Rev Neurosci.2019;
DOI: https://doi.org/10.1515/revneuro-2018-0080.

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Information Classification: General