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Arginine and Citrulline for the Treatment of MELAS Syndrome

Ayman W. El-Hattab, MD, FACMG[1], Mohammed Almannai, MD[2], and Fernando Scaglia, MD, FACMG[2]

Abstract

Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome is a maternally inherited mitochondrial disease with a broad spectrum of manifestations. In addition to impaired energy production, nitric oxide (NO) deficiency occurs in MELAS syndrome and leads to impaired blood perfusion in microvasculature that can contribute to several complications including stroke-like episodes, myopathy, and lactic acidosis. The supplementation of NO precursors, L-arginine and L-citrulline, increases NO production and hence can potentially have therapeutic utility in MELAS syndrome. L-citrulline raises NO production to a greater extent than L-arginine; therefore, L-citrulline may have a better therapeutic effect. The clinical effect of L-citrulline has not yet been studied and clinical studies on L-arginine, which are limited, only evaluated the stroke-like episodes' aspects of the disease. Controlled studies are still needed to assess the clinical effects of L-arginine and L-citrulline on different aspects of MELAS syndrome.

Keywords

stroke-like episodes, nitric oxide (NO), mitochondrial diseases, stable isotope, encephalomyopathy, lactic acidosis

Introduction

Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome is a maternally inherited mitochondrial disease that has a broad spectrum of manifestations, including stroke-like episodes, dementia, epilepsy, lactic acidosis, exercise intolerance, muscle weakness, migraine headaches, sensorineural hearing loss, peripheral neuropathy, recurrent vomiting, and short stature. Other less common manifestations include myoclonus, ataxia, optic atrophy, retinopathy, cardiomyopathy, diabetes, nephropathy, depression, anxiety, and psychotic disorders.[1] Clinically, the diagnosis of MELAS syndrome is based on the presence of 3 invariant criteria including stroke-like episodes before 40 years of age, encephalopathy characterized by seizures or dementia, and mitochondrial myopathy evident by lactic acidosis or ragged-red fibers. The clinical diagnosis is considered confirmed if there are also at least 2 of the 3 supportive criteria including normal early psychomotor development, recurrent headaches, and recurrent vomiting episodes.[2] Molecularly, MELAS syndrome is caused by different mutations in mitochondrial DNA, with the most common being the m.3243A>G mutation in the *MT-TL1* gene encoding the transfer $RNA^{Leu/(UUR)}$.[3] Childhood is the typical age of onset with approximately 70% of affected individuals presenting before 20 years. The disease rarely presents before 2 years or after 40 years of age.[4,5]

The pathogenesis of MELAS syndrome is not fully understood. However, the associated manifestations are likely due to several interacting mechanisms.[1] The m.3243A>G mutation results in impaired mitochondrial translation leading to decreased synthesis of mitochondrial proteins including the subunits of electron transport chain (ETC) complexes. This results in the impairment of ETC and energy production.[6] The inability of dysfunctional mitochondria to generate sufficient energy to meet the needs of various organs results in

[1] Division of Clinical Genetics and Metabolic Disorders, Pediatrics Department, Tawam Hospital, Al-Ain, United Arab Emirates
[2] Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA

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Corresponding Author:
Fernando Scaglia, MD, FACMG, Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, MS BCM225, Houston, TX 77030, USA.
Email: fscaglia@bcm.edu

the multiorgan dysfunction observed in MELAS syndrome. Energy deficiency can also stimulate mitochondrial proliferation in the smooth muscle and endothelial cells of small blood vessels leading to angiopathy that impairs blood perfusion in the microvasculature of several organs. Therefore, angiopathy can contribute to the complications observed in MELAS syndrome particularly the stroke-like episodes.[7] In addition to energy deficiency and angiopathy, there has been growing evidence that nitric oxide (NO) deficiency occurs in MELAS syndrome and can contribute significantly to its complications.[8]

There is no specific consensus approach for treating individuals with MELAS syndrome for which the management remains largely symptomatic.[1] Initially, a comprehensive evaluation is needed to assess the multiorgan involvement including neurological and cognitive evaluation, neuroimaging, audiologic and ophthalmologic examinations, growth assessment, echocardiogram and electrocardiogram, and screening for diabetes.[5] Complications can be managed by standard medical or surgical treatments, for example, cochlear implants for hearing loss and anticonvulsant drugs for epilepsy.[9] Nutrition support is needed for children failing to thrive, and rehabilitation with physical and occupational therapy is needed after stroke-like episodes. In addition, regular exercise can improve exercise capacity in individuals with MELAS syndrome and other mitochondrial myopathies.[10,11]

Several supplements are being used in MELAS syndrome based on limited clinical trials.[12] However, there is currently no clear evidence supporting the use of any supplementation in mitochondrial disorders.[13] Creatine, which is metabolized to phosphocreatine, is an essential phosphate donor for adenosine triphosphate (ATP) regeneration in muscle and brain that was shown to improve muscle strength and exercise activities in some individuals with MELAS syndrome.[14,15] Coenzyme Q_{10} (CoQ_{10}), which facilitates electron transfer and stabilizes the ETC complexes by providing protective antioxidant effects, can have beneficial effects on muscle weakness, fatigability, and lactate levels in some individuals with MELAS syndrome.[16] The CoQ_{10} does not cross the blood–brain barrier; therefore, it may have limited effect on the central nervous system. Idebenone is a CoQ_{10} analog that can cross the blood–brain barrier and has been shown to improve neurological complications in some case reports.[17] L-carnitine is required for the entry of long-chain fatty acids to the mitochondrial matrix where it undergoes β-oxidation. Therefore, L-carnitine supplementation can potentially enhance β-oxidation and replenish the intracellular pools of coenzyme A.[12]

As NO deficiency plays major roles in the pathogenesis of MELAS complications, supplementation of NO precursors, L-arginine and L-citrulline (henceforth arginine and citrulline, respectively), can be of therapeutic utility in this syndrome. In this article, we review the pathogenesis and consequences of NO deficiency in MELAS syndrome and evaluate the role of arginine and citrulline supplementation in treating individuals with this mitochondrial disease.

Nitric Oxide and MELAS Syndrome

Nitric oxide is synthesized from arginine by 3 NO synthase (NOS) isoforms—neuronal NOS (nNOS) primarily present in neuronal cells, endothelial NOS (eNOS) primarily present in endothelial cells, and cytokine-inducible NOS (iNOS) present in various cell types including macrophages, hepatocytes, and myocytes. The eNOS plays a role in regulating the physiological vascular tone, whereas iNOS produces NO under pathological conditions such as infections.[18] Nitric oxide synthase catalyzes the conversion of arginine to NO and citrulline. Citrulline can be recycled to arginine by the combined action of argininosuccinate synthase (ASS) and argininosuccinate lyase (ASL), which are expressed to some degree in nearly all cell types. Citrulline is a nonprotein amino acid whose main source is the *de novo* synthesis in the mitochondria of enterocytes.[19] Arginine is derived from the diet, as a result of protein turnover, and from the *de novo* synthesis from citrulline. The later accounts for $\sim 10\%$ of arginine production.[20,21] Both arginine and citrulline support NO synthesis in a variety of tissues including vascular endothelium, neurons, and macrophages.[22] The 3 enzymes responsible for recycling citrulline to produce NO (ASS, ASL, and NOS) have been shown to be coinduced and colocalized, suggesting that these proteins work as a complex functioning in the cellular compartmentalization of NO synthesis.[23-26]

There has been growing evidence that NO deficiency occurs in MELAS syndrome. Lower concentrations of nitrite and nitrate, NO metabolites, were found in individuals with MELAS syndrome during the stroke-like episodes.[27] Furthermore, NO synthesis rate, measured by stable isotope infusion techniques, was found to be lower in individuals with MELAS syndrome who are not experiencing acute stroke-like episodes.[28,29] Flow-mediated dilation (FMD), which is considered a measure of NO synthesized by endothelial cells in response to reperfusion, was found to be impaired in individuals with MELAS syndrome, providing further evidence for NO deficiency in this disease.[30,31] The etiology of NO deficiency in MELAS syndrome is believed to be multifactorial due to both impaired production and postproduction sequestration.[8]

Several factors can contribute to NO production impairment in MELAS syndrome. First, energy depletion due to mitochondrial dysfunction can stimulate mitochondrial proliferation in various tissues including vascular endothelial cells that can lead to impaired normal endothelial function, that is, endothelial dysfunction. Defective eNOS can reflect one aspect of endothelial dysfunction.[32] Second, decreased availability of NO precursors, arginine and citrulline, can have a major contribution in impaired NO production. Low-plasma arginine and citrulline were reported in individuals with MELAS syndrome.[28,29,32,33] Low-plasma citrulline may result from decreased citrulline synthesis in the mitochondria of enterocytes due to mitochondrial dysfunction.[32] Most of the synthesized citrulline is directed toward arginine synthesis; therefore, lower citrulline availability can result in decreased *de novo* arginine synthesis and lower intracellular arginine availability. In addition, individuals with MELAS syndrome were found to

have higher arginine clearance that may contribute to the lower plasma arginine observed in these individuals.[28] Third, decreased NO production can result from impaired NOS activity.[34] Excessive production of reactive oxygen species due to ETC dysfunction can result in the impairment of several cellular enzymes including NOS.[28] Additionally, the oxidative stress can lead to increased asymmetric dimethylarginine (ADMA), which is an endogenous inhibitor of NOS.[28] Finally, it has been proposed that NOS can be downregulated in mitochondrial dysfunction via a putative posttranscriptional mechanism. Nitric oxide plays a role in controlling oxidative phosphorylation through the inhibition of cytochrome c oxidase (COX). Therefore, such downregulation can be interpreted as a compensatory mechanism to improve oxidative phosphorylation in defective mitochondria.[34]

In addition to impaired NO production, postproduction NO sequestration can contribute to NO deficiency in MELAS syndrome. Mitochondrial proliferation in endothelial cells in MELAS syndrome can be associated with increased COX activity, which can react with and thus sequester NO.[7] In addition, oxidative stress can result in decreased NO availability by shunting NO into reactive nitrogen species formation.[31]

Decreased vascular endothelial NO availability can result in impaired perfusion in microvasculature of various tissues that can contribute to several complications in MELAS syndrome. Stroke-like episodes occur due to ischemic insults resulting from impaired perfusion in cerebral small blood vessels. Cerebral microvasculature angiopathy and NO deficiency have been suggested to be the main etiological factors causing these episodes.[7,27,32] Although the insufficient energy production explains the myopathy, NO deficiency can result in impaired muscular blood perfusion that may also have a significant contribution to the myopathic manifestations of MELAS syndrome.[8,35] Lactic acidosis in MELAS syndrome results from the inability of dysfunctional mitochondria to adequately oxidize glucose, leading to the accumulation of pyruvate and shunting of pyruvate to lactate.[7] Nitric oxide deficiency in MELAS syndrome can result in decreased blood perfusion that can aggravate lactic acidosis.[36]

Citrulline and Arginine Treatment in MELAS Syndrome

Both arginine and citrulline act as NO precursors; therefore, it was initially proposed that their administration can result in increased NO availability and hence have therapeutic benefits in stroke-like episodes in MELAS syndrome.[32] It was then shown in open-label trials that the administration of intravenous arginine to individuals with MELAS syndrome during stroke-like episodes led to improvement in the clinical symptoms associated with these episodes. Oral arginine supplementation during the interictal phase also decreased the frequency and severity of stroke-like episodes.[27,33] The therapeutic effect of arginine in stroke-like episodes in MELAS syndrome is proposed to be due to an increased NO availability, leading to improving cerebral vasodilation and blood flow. This potential mechanism has been supported by the demonstration that arginine supplementation to children and adults with MELAS resulted in increased NO production assessed by stable isotope infusion techniques.[28,29] Arginine supplementation has also resulted in improving the FMD.[30] The use of oral arginine as maintenance therapy and intravenous arginine during the stroke-like episodes has become commonly used in treating individuals with MELAS syndrome. During the acute stroke-like episode, it is recommended to give a bolus of intravenous L-arginine (500 mg/kg for children or 10 g/m^2 body surface area for adults) within 3 hours of symptom onset followed by the administration of similar dosage of intravenous arginine as continuous infusion over 24 hours for the next 3 to 5 days. Once an individual with MELAS has the first stroke-like episode, arginine should be administered prophylactically to reduce the risk of recurrent stroke-like episodes. A daily dose of 150 to 300 mg/kg/d oral arginine in 3 divided doses is recommended.[7,37]

The clinical effects of citrulline administration in MELAS syndrome have not yet been studied; however, stable isotope studies demonstrated that, similar to arginine supplementation, citrulline supplementation can increase NO production in adults and children with MELAS syndrome.[28,29] These isotope studies demonstrated that the increment in NO production associated with arginine and citrulline supplementation is accompanied with increments in both arginine flux and concentration indicating that the increment of NO production is driven by increased availability of arginine. Interestingly, citrulline supplementation was found to induce a greater increase in the NO synthesis rate than that associated with arginine supplementation, indicating that citrulline is a more effective NO precursor than arginine.[28,29] This can be explained by the greater ability of citrulline to make arginine available for NO synthesis as explained below.

Both arginine and citrulline have similar pharmacokinetic parameters except for C_{max} (maximum plasma concentration) that is several-folds higher for citrulline than for arginine indicating that citrulline has better absorption and systemic bioavailability than arginine.[38,39] The better bioavailability for citrulline can be due to higher intestinal absorption of citrulline relative to arginine resulting from the action of intestinal arginase II on ingested arginine and the ability of citrulline to bypasses the liver, whereas arginine is converted to ornithine in the liver through the action of arginase I.[19,23] Due to its higher bioavailability, citrulline supplementation results in increased arginine flux and plasma level more than supplementation of the same dose of arginine in children and adults with MELAS syndrome, leading to more arginine availability for NO synthesis.[28,29]

The greater ability of citrulline to increase arginine flux and arginine concentration explains part of its greater effect in increasing NO production due to its ability to make arginine more available for NO production. However, the more important feature of citrulline is its greater ability to increase the intracellular arginine pool in the subcellular compartment where NO is synthesized as explained in the following 3 points.[28,29] First, arginine transport across the cell membrane

is tightly regulated by the cationic amino acid transporter (CAT), whereas citrulline does not have a specific transporter.[19] Second, part of the intracellular arginine may be utilized by arginase, whereas citrulline acts as a direct precursor for intracellular arginine synthesis.[23] Third, citrulline is converted to arginine via the enzymes ASS and ASL that have been suggested to work as a complex with NOS.[23-26] Therefore, citrulline generates arginine in the subcellular compartments where NO synthesis takes place, so the *de novo-synthesized* arginine can be directly acted upon via NOS to produce NO. In contrast, plasma arginine needs to be transported into the cell and escape arginase degradation to finally reach the subcellular compartment that contains NOS.[28,29,40] Therefore, the *de novo-synthesized* arginine has been suggested to play a more important role in driving NO synthesis than plasma arginine. This would explain why citrulline led to a higher NO production than that associated with arginine supplementation.[28,29,40]

Increasing NO availability with arginine or citrulline supplementation can potentially improve perfusion in all microvasculature compartments. Therefore, the effect of arginine and citrulline supplementation may not be limited to improving stroke-like episodes but may also lead to improvements in other clinical features of MELAS syndrome, including muscle weakness, exercise intolerance, and lactic acidosis. Interestingly, arginine and citrulline supplementation has been reported to result in a reduction in plasma alanine and lactate concentrations, suggesting that such supplementation may improve lactic acidemia in MELAS syndrome by increasing NO production and improving perfusion and oxygen delivery.[28,36]

Based on the finding that citrulline supplementation can result in a higher NO production than arginine supplementation, it was proposed that citrulline may have a better therapeutic effect than arginine.[28,29] However, research evaluating the clinical effect of arginine and citrulline supplementation in MELAS syndrome is very limited, with the effect of arginine on stroke-like episodes being the only issue addressed to date.[27,33] Therefore, additional measures of the clinical effects of arginine and citrulline supplementation on different aspects of MELAS syndrome are warranted to determine the potential therapeutic effect of such supplementation.

Conclusion

As NO deficiency can play a major role of the pathogenesis of MELAS syndrome complications, the supplementation of NO precursors, arginine and citrulline, can result in an increased NO availability and hence may have therapeutic effects on NO deficiency-related manifestations of MELAS syndrome. Citrulline supplementation can raise NO production to a greater extent than that associated with arginine. Therefore, citrulline supplementation may have a better therapeutic effect than arginine. However, the clinical effect of citrulline has not yet been studied and the clinical studies on arginine are limited and only evaluated the stroke-like episodes' aspects of the disease. Controlled studies assessing the clinical effects of arginine and citrulline supplementation on different aspects of MELAS syndrome are needed to further support the use of such supplementation as a treatment modality for MELAS syndrome and compare the clinical effect of citrulline to that of arginine.

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Declaration of Conflicting Interests

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