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Organelles in focus

Mitochondria: Role of citrulline and arginine supplementation in MELAS syndrome

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ABSTRACT

Mitochondria are found in all nucleated human cells and generate most of the cellular energy. Mitochondrial disorders result from dysfunctional mitochondria that are unable to generate sufficient ATP to meet the energy needs of various organs. Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome is a frequent maternally inherited mitochondrial disorder. There is growing evidence that nitric oxide (NO) deficiency occurs in MELAS syndrome and results in impaired blood perfusion that contributes significantly to several complications including stroke-like episodes, myopathy, and lactic acidosis. Both arginine and citrulline act as NO precursors and their administration results in increased NO production and hence can potentially have therapeutic utility in MELAS syndrome. Citrulline raises NO production to a greater extent than arginine, therefore, citrulline may have a better therapeutic effect. Controlled studies assessing the effects of arginine or citrulline supplementation on different clinical aspects of MELAS syndrome are needed.

Organelle facts

- Mitochondria generate most of cellular energy.
- Mitochondrial disorders result from dysfunctional mitochondria that are unable to generate sufficient ATP to meet the energy needs of various organs.
- MELAS syndrome is a maternally inherited mitochondrial disease characterized by encephalomyopathy, lactic acidosis, and stroke-like episodes.
- NO deficiency occurs in MELAS and can contribute to its complications.
- Arginine and citrulline act as NO precursors and their administration results in increased NO production and can have therapeutic utility in MELAS.
- Citrulline can raise NO production to a greater extent than arginine and may have a better therapeutic effect.

1. Introduction

Mitochondria are organelles found in all nucleated human cells and perform a variety of essential functions, including the generation of most cellular energy. Mitochondria are composed of two bilayer membranes that create two distinct compartments; an intermembrane space and a matrix space within the inner membrane. Whereas the mitochondrial outer membrane contains protein complexes that are needed for numerous functions, including protein import, metabolite and ion flux, and pro- and anti-apoptotic factors, the inner mitochondrial membrane harbors the electron transport chain complexes that transfer electrons, translocate protons, and produce ATP. Mitochondria contain extra-chromosomal DNA (mitochondrial DNA, mtDNA), however, only a very small proportion of mitochondrial proteins (13 out of approximately 1500) are encoded by mtDNA. The majority of mitochondrial proteins are encoded by the nuclear DNA (nDNA). Since mtDNA is maternally inherited, this dual genome pattern of inheritance gives rise to both Mendelian traits and matrilineal traits. Defects in either mtDNA or nDNA-encoded mitochondrial proteins can result in mitochondrial dysfunction leading to mitochondrial diseases. In addition to a wide range of cellular perturbations such as altered calcium homeostasis, alterations in signal transduction, and dysregulated apoptosis, dysfunctional mitochondria are unable to generate sufficient ATP to meet the

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energy needs of various organs, particularly those with high energy demand, including the nervous system, skeletal and cardiac muscles, kidneys, liver, and endocrine systems. Disturbed mitochondrial function in various organs can explain the multi-organ manifestations of mitochondrial diseases that include epilepsy, intellectual disability, skeletal and cardiac myopathy, sensorineural hearing loss, endocrine diseases, and renal impairment. Defects in nDNA genes can be inherited in an autosomal recessive, autosomal dominant, or X-linked manner, whereas, by an as yet unknown mechanism, mtDNA is strictly maternally inherited. While nDNA is composed of approximately 3 billion nucleotides per haploid genome, mtDNA is composed of 16,569 nucleotides, albeit in multiple copies, ranging from hundreds to thousands per cell. Given the large number of nDNA-encoded proteins found in mitochondria, the majority of mitochondrial diseases would be expected to be Mendelian, however, recurrent mutations arising in mtDNA also account for a large number of cases, both sporadic and familial (Wallace, 1999).

Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome is one of the most frequent maternally inherited mitochondrial disorders, with an estimated prevalence of 60:100,000 (Chinnery and Turnbull, 2001). It is caused by different mutations in mtDNA, with the most common being the m.3243A>G mutation in the *MTTL1* gene that encodes $tRNA^{Leu/(UUR)}$ (Wallace, 1992). MELAS syndrome has a broad spectrum of manifestations, including stroke-like episodes, exercise intolerance, muscle weakness, epilepsy, dementia, migraine headaches, short stature, sensorineural hearing loss, and lactic acidosis. MELAS syndrome typically affects a young population, with 75% of cases presenting before 20 years of age, and causes significant morbidity, with the life-time prevalence of stroke-like episodes approaching 99% (Hirano and Pavlakis, 1994). No specific consensus has been established for the treatment of MELAS syndrome, with several medications being used, including antioxidants and cofactors, that have no proven efficacy (Scaglia and Northrop, 2006).

Energy depletion due to mitochondrial dysfunction can explain many of the multi-organ manifestations of MELAS syndrome. In addition, there has been growing evidence that nitric oxide (NO) deficiency occurs in MELAS syndrome and can contribute significantly to its complications (El-Hattab et al., 2012a; Koga et al., 2005, 2006, 2007; Naini et al., 2005; Sproule and Kaufmann, 2008; Tengan et al., 2007; Vattemi et al., 2011). NO produced by vascular endothelium plays a major role in vascular smooth muscle relaxation that is needed to maintain the patency of small blood vessels (Green et al., 2004; Toda and Okamura, 2003). Therefore, NO deficiency in MELAS syndrome can result in impaired blood perfusion in the microvasculature of different organs that potentially contributes to the pathogenesis of several complications including stroke-like episodes, myopathy, and lactic acidosis. The amino acids arginine and citrulline act as NO precursors, and their administration has been shown to restore NO production in MELAS syndrome. Therefore, arginine and citrulline may be of therapeutic value in this syndrome (El-Hattab et al., 2012a, 2012b).

In this article, we review NO metabolism, discuss the pathogenesis and consequences of NO deficiency in MELAS syndrome, and evaluate the role of arginine and citrulline supplementation in treating NO deficiency in MELAS syndrome.

2. Overview of nitric oxide synthesis in physiologic state

NO is synthesized from arginine by three 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, muscles, and chondrocytes. eNOS plays a role in regulating the physiological vascular tone, whereas iNOS produces NO under pathological conditions, *e.g.* infection (Förstermann et al., 1994; Villanueva and Giulivi, 2010). NOS, in conjunction with tetrahydrobiopterin and oxygen, 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. Therefore, both arginine and citrulline support NO synthesis in a variety of tissues (Wu and Morris, 1998) (Fig. 1).

Arginine is derived from the diet, as a result of protein turnover, and from endogenous (*de novo*) synthesis from citrulline. Arginine is utilized in protein synthesis as well as for the synthesis of urea, NO, creatine, agmatine, and polyamines (Morris, 2007). Citrulline is a non-protein amino acid that functions as an intermediate in the urea cycle and as a precursor of arginine. The main source of citrulline is the *de novo* synthesis in the small intestine where all of the enzymes involved in its synthesis are located in the enterocyte mitochondria (Curis et al., 2005; Moinard and Cynober, 2007). The activities of the two enzymes that catabolize citrulline, ASS and ASL, are very low in the intestine. Therefore, citrulline cannot be catabolized in enterocytes and is released into the circulation (Husson et al., 2003). The majority of citrulline released by the intestine is metabolized within the kidney, where it is converted into arginine. Therefore, endogenous arginine synthesis involves an inter-organ pathway known as the intestinal-renal axis, with *de novo* arginine synthesis from citrulline representing ~10% of arginine production (Castillo et al., 1993; Dhanakoti et al., 1990). Circulating arginine can be catabolized by liver arginase I to fuel the urea cycle (Wu and Morris, 1998) (Fig. 1).

Both arginine and citrulline support NO synthesis in a variety of tissues including vascular endothelium, neurons, and macrophages. The three enzymes responsible for recycling citrulline to produce NO (ASS, ASL, and NOS) have an interesting relationship. It has been demonstrated that ASS and ASL are transcriptionally co-induced with iNOS in various cell types (Mori and Gotoh, 2000; Oyadomari et al., 2001). Furthermore, ASS and ASL interact and co-localize with the different NOS isoforms, suggesting that these proteins work as a complex (Erez et al., 2011; Flam et al., 2001; Solomonson et al., 2003). The loss of ASL was shown to result in decreased abundance of the ASL–ASS–NOS complex and NO synthesis, suggesting that the formation of this complex is needed for NO production and may function in the cellular compartmentalization of NO synthesis (Erez et al., 2011).

NO synthesis is largely dependent upon the availability of intracellular arginine that is affected by the transport of extracellular arginine, the intracellular synthesis of arginine from citrulline, and the activity of arginase. Arginine is transported into the cells *via* cationic amino acid transporter (CAT) isoforms CAT-1, CAT-2, and CAT-3. CAT-1 is expressed ubiquitously, CAT-2 is highly expressed in the liver and at less abundant levels in various tissues, while CAT-3 expression is limited to the brain. The expression of these transporters has been shown to be co-induced with iNOS in a wide variety of cells, indicating that arginine transport capacity increases to support elevated rates of NO synthesis. Arginase exists in two isoforms: cytosolic arginase I, which is highly expressed in the liver, and mitochondrial arginase II, which is expressed in non-hepatic organs including the kidney, brain, and small intestine. Both NOS and arginase use arginine as a common substrate, and arginase may reduce NO production by competing with NOS for arginine (Mori and Gotoh, 2000; Wu and Morris, 1998).