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# Disruption of mitochondrial homeostasis in organic acidurias: insights from human and animal studies

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**Abstract** Organic acidurias or organic acidemias constitute a group of inherited disorders caused by deficient activity of specific enzymes of amino acids, carbohydrates or lipids catabolism, leading to large accumulation and excretion of one or more carboxylic (organic) acids. Affected patients usually present neurologic symptoms and abnormalities, sometimes accompanied by cardiac and skeletal muscle alterations, whose pathogenesis is poorly known. However, in recent years growing evidence has emerged indicating that mitochondrial dysfunction is directly or indirectly involved in the pathology of various organic acidemias. Mitochondrial impairment in some of these diseases are generally due to mutations in nuclear genes of the tricarboxylic acid cycle or oxidative phosphorylation, while in others it seems to result from toxic influences of the endogenous organic acids to the mitochondrion. In this minireview, we will briefly summarize the present knowledge obtained from human and animal studies showing that disruption of mitochondrial homeostasis may represent a relevant pathomechanism of tissue damage in selective organic acidemias. The discussion will focus on mitochondrial alterations found in patients affected by organic acidemias and by the deleterious effects of the accumulating organic acids on mitochondrial pathways that are crucial for ATP formation and transfer. The elucidation of the mechanisms of toxicity of these acidic compounds offers new perspectives for potential novel adjuvant therapeutic strategies in selected disorders of this group.

**Keywords** Organic acidemias · Organic acids · Mitochondrial dysfunction · Brain damage

**Abbreviations**

| | |
|---|---|
| Acyl-CoA | Acyl coenzyme A |
| ATP | Adenosine triphosphate |
| CSF | Cerebrospinal fluid |
| DNA | Deoxyribonucleic acid |
| D-2-OHG | D-2-Hydroxyglutarate |
| D-2-OHGA | D-2-Hydroxyglutaric aciduria |
| EE | Ethylmalonic encephalopathy |
| FADH2 | Flavin adenine dinucleotide reduced |
| GAI | Glutaric acidemia type I |
| L-2-OHG | L-2-Hydroxyglutarate |
| L-2-OHGA | L-2-Hydroxyglutaric aciduria |
| MMA | Methylmalonic acidemia |
| MPT | Mitochondrial permeability transition |
| mtDNA | Mithocondrial DNA |
| MOHBA | 2-Methyl- 3-hydroxybutyric aciduria |
| MHBA | 3-Methylglutaconic aciduria |
| MRI | Magnetic Ressonance Imaging |
| NADH | Nicotinamide adenine dinucleotide reduced |
| OAs | Organic acidemias |
| OXPHOS | Oxidative phosphorylation |
| PPA | Propionic acidemia |
| ROS | Reactive oxygen species |
| TCA | Tricarboxylic acid |

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## Introduction

Organic acidemias (OAs) are inherited metabolic disorders biochemically characterized by tissue accumulation and urinary excretion of large amounts of organic acids. They are caused by deficiency of enzymatic activities in the catabolism of amino acids, carbohydrates or lipids (Goodman 1980; 1981; Chalmers and Lawson 1982; Scriver et al. 2001). The common clinical presentation of OAs is that of a toxic encephalopathy, with seizures, hypotonia, lethargy, coma, vomiting, respiratory distress and poor feeding in the neonatal period. Clinical features of some OAs may occur in the older child or adolescence with progressive neurologic deterioration, loss of intellectual function, ataxia, Reye syndrome, recurrent ketoacidosis or psychiatric symptoms. Neuropathological abnormalities of the basal ganglia, as well as white matter changes (hypo/demyelination) and macrocephaly are relatively common in the affected individuals. There is a group of OAs whose symptoms are nearly exclusively neurologic, being called cerebral "organic acidemias" and include glutaric acidemia type I, L-2-hydroxyglutaric aciduria, D-2-hydroxyglutaric aciduria, 4-hydroxybutyric aciduria and others (Hoffmann et al. 1993; Kolker et al. 2006).

Although ketoacidosis, hyperammonemia, hyperglycinemia, lactic acidemia, hypoglycemia and neutropenia are common laboratory findings in some OAs, diagnosis is essentially made by demonstrating characteristic elevations of certain organic acids in urine (Sweetman 1991; Kamboj 2008). Outcome is often improved by early diagnosis and treatment. Therapy, often imperfect at best, include dietary manipulation to reduce enzyme substrate, administration of appropriate vitamins to increase residual activity of the mutant enzyme, increase the excretion of potentially toxic metabolites by providing glycine and/or L-carnitine and, in some cases, by supplying enzyme in the form of liver transplantation. In some conditions, like glutaric acidemia type I, it is important to avoid or promptly treat ketotic episodes that cause acute CNS damage during infections and other stress catabolic situations with adequate supply of calories especially from carbohydrates and lipids and with low protein to support anabolism. The objective of treatment is to restore the biochemical and physiologic homeostasis. Some disorders are easily treated if promptly diagnosed and treated, but in others functional deterioration of brain and other organs with high energy demands are quite common. This is particularly true for methylmalonic acidemia and propionic acidemia, in which neither diet, vitamin therapy, or liver transplantation appear to prevent neurologic complications such as leucoencephalopathy and basal ganglia atrophy (Leonard et al. 2001). For these and some other OAs, it has been suggested that the formation of the toxic metabolites may occur within the brain (Hoffmann et al. 1993; Kolker and Okun 2005).

Because most intermediates that accumulate in OAs are produced within mitochondria, where they reach their highest intracellular concentration before leaving the cell to be excreted in urine, it is possible that disease pathogenesis is due to impairment of oxidative phosphorylation (OXPHOS) and other mitochondrial pathways involved with energy production. In this context, there is growing evidence that the accumulated coenzyme A esters and organic acids are toxic not only to brain, but also to heart, skeletal muscle, liver, kidney, pancreas, retina and other tissues. Indeed, data from human and animal studies indicate that part of this toxicity is due to energy deficiency, decisively contributing to the clinical syndrome in selected OAs. Respiratory chain enzyme deficiencies seen in tissues from patients with propionic acidemia, methylmalonic acidemia and other OAs suggest secondary mitochondrial damage, whereas defects of pyruvate dehydrogenase, of the tricarboxylic acid cycle (TCA) and respiratory chain (RC) are considered primary causes of mitochondrial dysfunction. Furthermore, since acyl-CoA derivatives often react with L-carnitine, L-carnitine deficiency may further impair mitochondrial function.

In a previous review we discussed disruption of redox homeostasis by organic acids accumulating in various organic acidemias as a pathogenic mechanism underlying the brain damage (Wajner et al. 2004a). This review will briefly summarize the mounting evidence that mitochondrial dysfunction occurs in some OAs, and that it may be a significant cause of tissue damage in these disorders.

## Mitochondrial disease

Mitochondria are subcellular organelles that perform crucial biochemical functions needed for cellular homeostasis and survival, including oxidative phosphorylation (OXPHOS) and tricarboxylic acid (TCA) cycle, regulation of redox and calcium signaling, fatty acid oxidation and iron metabolism in eukaryotic cells (McBride et al. 2006 for review). They also play an important role in the regulation of apoptosis in response to extracellular and intracellular signals (Büeler 2010).

OXPHOS is the major source of cellular ATP and reactive oxygen species (ROS) (Lemasters et al. 1999). These reactive species can cause oxidative damage in mitochondrial components such as lipids, proteins and DNA in a cascade that may lead to apoptotic or necrotic cell death (Kroemer and Reed 2000). Oxidative damage and high intracellular calcium lead to mitochondria stress and collapse of internal membrane potential in a process called mitochondrial permeability transition (MPT) (Kowaltowski et al. 2009). As a result of MTP activation, $NAD^+$ is released from mitochondria into the cytosol,

leading to depletion of NAD(H) pool and bioenergetic failure. Pharmaceutical and genetic approaches have confirmed the important role of MTP in mechanisms leading to cell death.

Brain, cardiac and skeletal muscle are tissues highly dependent on OXPHOS for energy production and therefore very vulnerable to disordered mitochondrial function. Regarding to neurons, the high energy requirements of these cells needed to maintain the resting membrane potential, which is critical for their function, depends almost entirely on OXPHOS and on the activity of ATPases which modulate ion flux (Martin et al. 1994). When OXPHOS is impaired in these cells by a respiratory chain defect, the production of free radicals increases causing further cell damage. It is therefore expected that defects of energy production should be characterized by alterations in tissues with high energy demand, such as the central nervous system (encephalopathy, movement disorders and developmental retardation), skeletal muscle (myopathy), heart (cardiopathy) and others.

Over the last twenty years there has been an increasing recognition of the role played by the mitochondrion in causing human diseases (Leonard and Schapira 2000a,b; Schapira 2006, 2008). Mitochondrial diseases can be caused by mutations of mitochondrial DNA or of nuclear genes that encode mitochondrial proteins, or by endogenous and exogenous toxic compounds that target mitochondrial metabolism. Defects of the OXPHOS system due to defects in either the nuclear or mitochondrial genomes are considered primary disorders of mitochondrial function. Secondary disorders of mitochondrial function are those caused by endogenous or exogenous toxins.

It is well recognized that mitochondrial dysfunction commonly results in brain damage. Defects of OXPHOS enzyme activities and mutations of mtDNA have been identified in a number of neurodegenerative pathologies including Alzheimer disease and Parkinson disease and in other less common disorders such as the inherited OAs. The major question is whether they represent primary defects or rather abnormalities due to other factors indirectly related to pathogenesis.

It has been recognized over the last two decades that numerous toxins inhibit mitochondrial function. MPTP, a selective complex I inhibitor, induces parkinsonism in animals and humans and this is paralleled by complex I inhibition found in the substantia nigra of patients with Parkinson disease. Furthermore, 3-nitropropionic acid, a contaminant of mildewed sugar cane and an irreversible inhibitor of complex II activity, and malonate, a competitive inhibitor of this complex, cause striatum pathology identical to that of Huntington disease. Interestingly, these disorders with alterations of OXPHOS have striatal degeneration that is commonly seen in various OAs.

## Primary mitochondrial dysfunction in OAs

OAs with primary mitochondrial dysfunction are biochemically characterized by accumulation of lactic acid in tissues, blood, urine and sometimes CSF. Some patients also excrete increased quantities of organic acid intermediates of the TCA cycle. Thus, individuals with fumaric aciduria due to fumarase deficiency excrete large amounts of fumaric acid and lactate as well.

Primary mitochondrial dysfunction occurs fundamentally in inherited defects of the pyruvate metabolism, the TCA cycle and the respiratory chain. Pyruvate can be converted to acetyl-CoA by the pyruvate dehydrogenase complex, with subsequent oxidation in the TCA cycle, or to oxalacetate by pyruvate carboxylase, which enters the gluconeogenic pathway. The TCA cycle has as its primary function the generation of reducing equivalents in the form of NADH and $FADH_2$ used by the respiratory chain to produce energy in the form of ATP. A few rare human diseases are known to be caused by mutations of nuclear genes encoding enzymes of the TCA cycle and most of these affect the neuromuscular system (Rustin et al. 1997). The few isolated primary TCA cycle defects biochemically characterized to date include deficiencies of $\alpha$-ketoglutarate dehydrogenase, succinate dehydrogenase and fumarase.

Clinical manifestations of disorders of pyruvate metabolism (pyruvate carboxylase deficiency, pyruvate dehydrogenase deficiency and pyruvate transporter defect) and of the TCA cycle include encephalopathy, typical Leigh syndrome, myopathy, hepatopathy, cardiomyopathy, growth retardation, hypotonia, failure to thrive, lactic acidemia and lactic aciduria - sometimes with hyperalaninemia or hypoglycemia. Signs and symptoms may be precipitated or accentuated by metabolic or infectious stress.

The respiratory chain oxidizes NADH and FADH2 with concomitant energy transduction from the electron flux into ATP. Disorders of mitochondrial OXPHOS, although individually rare, collectively have a high prevalence relative to other inborn errors of metabolism (1/5,000) (Smeitink et al. 1998). Although variable in cause and clinical features (Thorburn 2004a, 2004b), they are thought to be relatively frequent causes of metabolic abnormality in neuropediatrics, presenting with failure to thrive, as well as hepatic, cardiac, renal, gastrointestinal, endocrine, hematological or other symptoms (Munnich et al. 1996). Disease-causing mutations are recognized in more than 30 of the 37 mtDNA genes and in more than 30 nuclear genes. Most OXPHOS disorders in adults are caused by mtDNA mutations, while children are more likely to have nuclear-encoded disorders. Due to the ubiquitous nature of OXPHOS, respiratory chain defects should be considered in patients with an association of neuromuscular and non-neuromuscular symptoms, with a rapidly progressive

course or with multi-organ disease. Biochemically, they are characterized by an increase in the concentrations of reducing equivalents (NADH and FADH2) in both mitochondria and cytoplasm with high plasma lactate levels and an impairment of the TCA cycle functioning.

## Secondary mitochondria dysfunction in OAs

Patients with OAs often show features of mitochondrial disorders such as basal ganglia abnormalities, intermittent or persistent increase of lactate in plasma, urine, CSF and brain similar to what is seen in Leigh syndrome, as well as reduced activity of respiratory chain complexes and abnormal mitochondrial morphology on light or electron microscopy. In addition, there is a large body of experimental evidence showing that organic acids that accumulate in some OAs inhibit bioenergetics in brain and other high energy dependent tissues, suggesting secondary mitochondrial impairment in these conditions. It should be stressed that the central nervous system is very susceptible to toxic damage during infancy and childhood when the brain has an exceptionally high energy demand. The vulnerability of cardiac and skeletal muscle is also high, and these tissues suffer especially during crises of metabolic decompensation.

We therefore suggest that mitochondrial dysfunction may be relevant to the pathogenesis of some OAs, and a discussion of some of these disorders follows.

## Methylmalonic acidemia (MMA)

MMA is caused by deficiency of L-methylmalonyl-CoA mutase, is relatively frequent (1:48,000 newborns), and is characterized by accumulation and excretion of methylmalonate, metabolites of propionyl-CoA and, during periods of metabolic decompensation, ammonia and lactate (Fenton et al. 2001). Patients present predominantly with neurological signs and symptoms. Observations on MMA patients and a mut-knock out mouse model of the disease, as well as *in vivo* and *in vitro* experimental data, strongly support the view that mitochondrial dysfunction is common in this condition (Chandler et al. 2009; Valayannopoulos et al. 2009). We are not expanding this discussion here showing the observations supporting this conclusion because another paper published in this issue by Melo and collaborators (2010) will deal with these aspects in details.

## Propionic acidemia (PPA)

PPA is caused by deficiency of propionyl-CoA carboxylase activity, which leads to accumulation and excretion of propionate, 3-hydroxypropionate, methylcitrate and propionylglycine, as well as ammonia and lactate, especially during metabolic crises (Fenton et al. 2001). Neuropathological features include demyelination, cerebral atrophy and abnormalities of the putamen and caudate. Cardiomyopathy, which is a frequent complication of this disorder, and the neurological features have been attributed to organic acid accumulation because various propionyl-CoA metabolites have been shown to inhibit pathways of energy production (Cheema-Dhadli et al. 1975; Gregersen 1981; Evangeliou et al. 1985; Massoud and Leonard 1993; Okun et al. 2002; Baumgartner et al. 2007). Abnormal mitochondrial structure has been demonstrated in skeletal muscle, heart and liver from propionic acidemia patients, together with severe impairment of OXPHOS manifested by a marked decrease of the production of ATP and phosphocreatine and of the expression, activity and amount of mitochondrial DNA of the electron transfer complexes I–IV (Mardach et al. 2005; Schwab et al. 2006; Keyzer et al. 2009). Further, the biochemical abnormalities found during acute metabolic crises, including increased plasma, CSF and brain lactate concentrations found on the magnetic resonance spectroscopy, as well as ketonemia and hypoglycemia (Chemelli et al. 2000) suggest the involvement of mitochondrial dysfunction in pathogenesis.

Complementary *in vitro* and *in vivo* studies have shown that propionyl-CoA inhibits the activities of the pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, and the respiratory chain complex III, whereas propionate decreases the uptake of ketones into brain and synaptic Na+,K+-ATPase activity (Dutra et al. 1991; Wyse et al. 1998; Schwab et al. 2006; Keyzer et al. 2009). Furthermore, propionate strongly inhibits oxygen consumption and pyruvate and α-ketoglutarate oxidation in rat liver mitochondria at concentrations found in the plasma of patients with PPA (Stumpf et al. 1980; Gregersen 1981). It has been also proposed that, because the enzyme defect blocks anaplerotic biosynthesis of succinyl-CoA from propionyl-CoA, there is diminished TCA cycle activity (Brunengraber and Roe 2006). This may be especially significant for the heart and skeletal muscle, in which propionate is a major anaplerotic substrate. The TCA cycle plays an important role in the normal contractility of cardiac muscle (Russell et al. 1995; Gibala et al. 2000). It is therefore likely that secondary mitochondrial dysfunction induced by accumulated toxic metabolites plays a role in the pathogenesis of PPA, as it does in MMA.

## 2-methyl-3-hydroxybutyric aciduria (MOHBA)

Eleven patients have been described with MOHBA (García-Villoria et al. 2009), a rare autosomal recessive disorder

caused by deficiency of 2-methyl-3-hydroxybutyryl-CoA dehydrogenase and characterized by hyperlacticacidemia and neurologic dysfunction with basal ganglia abnormalities. The organic acid profile and the persistent increase of plasma and occasionally CSF and brain lactate concentrations found in affected patients (Zschocke et al. 2000; Ensenauer et al. 2002) are attributed to a secondary deficiency of complexes I and IV of the respiratory chain that mimic a mitochondrial disease (Perez-Cerda et al. 2005; Olpin et al. 2002; García-Villoria et al. 2009). In addition, 2-methyl-3-hydroxybutyric acid has been shown to inhibit $CO_2$ production from acetate, citrate and glucose, as well as the activities of the respiratory chain complex IV and mitochondrial creatine kinase in rat brain, indicating a blockage in TCA and of electron flux through the respiratory chain (crucial pathways for most cellular energy production) and of ATP transfer and buffering, respectively (Rosa et al. 2005). Taken together, these observations indicate that aerobic energy metabolism is inhibited by 2-methyl-3-hydroxybutyric acid, a fact that may be related to disease pathogenesis and to lactic acidemia in these patients.

### 3-methylglutaconic aciduria (MHGA)

MHGA is characterized by increased urinary excretion of 3-methylglutaconic, 3-methylglutaric and (sometimes) 3-hydroxyisovaleric acids, and is very heterogeneous, there being at least five distinct types. Observations on patients with types II and IV strongly suggest that they exhibit mitochondrial dysfunction (Barth et al. 1999; Scaglia et al. 2001; Wortmann et al. 2009).

Type II MHGA, also known as Barth syndrome or X-linked 3-methylglutaconic aciduria, is present in about 1 in 250,000 live births (Mazzocco et al. 2007), and is caused by mutations of the TAZ gene (Marzilliano et al. 2007). The activity of 3-methylglutaconyl-CoA hydratase is normal. Patients with Type II MHGA show dilated cardiomyopathy, skeletal myopathy, neutropenia, growth retardation and intermittent lactic acidemia with mitochondrial abnormalities in the heart and skeletal muscle, and variable deficiency of various respiratory chain complexes. The concentrations of cytochromes $c_1$ + c, b and aa3 are reduced, suggesting decreased mitochondrial stability (Barth et al. 1983, 1996, 1999).

Over 120 patients with type IV disease, also called the "unclassified" form, have been reported (Wortmann et al. 2009). They show mild 3-methylglutaconic aciduria, normal activity of 3-methylglutaconyl-CoA hydratase, and clinical heterogeneity with progressive neurologic impairment, including Leigh-like encephalomyopathy, variable psychomotor retardation, hypertonicity, hypotonia, optic atrophy, dysmorphic features, seizures, cardiomyopathy and hepatic dysfunction. While the primary defect is not known, biochemical features of dysfunctional oxidative phosphorylation, including lactic acidemia, complex V and I deficiencies (Sperl et al. 2006) and mitochondrial abnormalities on light microscopy are common (Wortmann et al. 2009). Reduced glucose uptake by brain has been demonstrated by PET scans in four patients (Al-Essa et al. 1999).

### Glutaric aciduria type I (GA I)

GA I is a neurometabolic disorder caused by deficiency of glutaryl-CoA dehydrogenase activity, which leads to accumulation of glutarate and 3-hydroxyglutarate (Goodman et al. 1975, 1977). The incidence of this disorder is about 1:30,000 to 1:100,000 newborns (Lidner et al. 2004). Most untreated patients develop encephalopathic crises that result in striatal necrosis and progressive cortical injury. Although the pathogenesis of neuronal damage is obscure, a mitochondrial role is suggested by the findings of lactic acidemia in some patients, increased lactate concentrations in the white matter in one patient brain and prevention of striatal destruction by aggressive therapy with high doses of glucose during crises of metabolic decompensation (Strauss and Morton 2003; Bodamer et al. 2004; Kolker et al. 2004a, 2006).

Observations made in the GA I genetic knockout mice model show enlarged and desintegrated mitochondria as one of the earliest pathologic events in the cerebral cortex in postsynaptical regions with edema of dendritic spines after lysine or protein overload, which were paralleled by worsening symptoms and progressive encephalopathy (Zinnanti et al. 2007). Indeed, the high lysine or protein diet provoked vasogenic oedema and blood–brain barrier breakdown within the striatum in weaning mice, which were accompanied by glutaric acid brain accumulation, neuronal loss, hemorrhages, paralysis seizures and death (Zinnanti et al. 2006). Most adult mice survive the lysine overload, but developed bilateral striatal degeneration, with neuronal loss, axonal swelling, myelin disruption and gliosis in the striatum and cerebral cortex. Finally, it was also found a significant reduction of ATP, phosphocreatine and alpha-ketoglutarate in the brain of these genetically modified animals. Other investigators proposed that mitochondrial dysfunction represents a major pathomechanism of striatal injury in GA I, similarly to what is observed in infants with hypoxia-ischemia (reversible striatal abnormalities) or systemic intoxication with 3-nitropropionic acid (permanent striatal destruction), an irreversible inhibitor of complex II activity of the respiratory chain (Strauss and Morton 2003).

While there is abundant evidence that excitototixity and oxidative stress are important pathomechanisms of brain damage in GA I (Kolker et al. 2001; Marques et al. 2003; Kolker et al. 2004a, 2004b; Wajner et al. 2004b; Ferreira et al. 2005; Latini et al. 2005a,b; Sauer et al. 2005; Latini et al. 2007; Sauer 2007; Oliveira et al. 2008), there is also experimental evidence that mitochondrial function may be impaired by glutaryl-CoA, glutaric acid and 3-hidroxyglutaric acid. Therefore, the relevance of bioenergetic impairment on the pathogenesis of this disorder is not yet understood and other pathomechanisms should be considered (Goodman 2004).

## D-2-hydroxyglutaric aciduria (D-2-OHGA)

About 80 patients have been described with D-2-OHGA, a neurometabolic organic acidemia with variable clinical expression caused by a deficiency of D-2-hydroxyglutarate dehydrogenase (Struys 2006; Kranendijk et al. 2009). The disorder is characterized biochemically by the accumulation of D-2-hydroxyglutarate (D-2-OHG) in CSF, blood and urine and clinically by epilepsy, cardiomyopathy, hypotonia and facial dysmorphic features. Many patients show increased urinary excretion of $\alpha$ -ketoglutaric acid, succinate and lactate, while others excrete increased amounts of dicarboxylic acids (Van der Knaap et al. 1999; Muntau et al. 2000; Misra et al. 2005; Struys 2006), perhaps reflecting a secondary disturbance of the citric acid cycle or respiratory chain dysfunction. It was also reported a case of D-2-OHGA with the same biochemical profile and cerebral MRI abnormalities suggestive of a mitochondrial disorder (Wajner et al. 2002).

Animal studies have shown that D-2-OHG strongly reduces complex V (ATPase) activity in submitochondrial particles from bovine heart, as well as complex IV activity (cytochrome oxidase) in homogenates from rat brain and human skeletal muscle, indicating decreased electron flux through the respiratory chain and impaired energy production (da Silva et al. 2002; Kolker et al. 2002; Latini et al. 2005c). It is feasible that D-2-OHG-induced inhibition of the respiratory chain leads to a secondary blockage of the TCA, probably by increasing the NADH/NAD+ and FADH2/FAD ratios, finally resulting in an increase of lactate and of the intermediates of this cycle. D-2-OHG has also been shown to inhibit the activity of creatine kinase, which is critical for intracellular ATP transfer, in rat brain, heart and skeletal muscle (da Silva et al. 2003a, 2004). These observations suggest that energy cell production and transfer is disturbed in D-2-OHGA, and may in part explain the encephalopathy, myopathy and cardiomyopathy, as well as lactic acidemia in some patients.

## L-2-hydroxyglutaric aciduria (L-2-OHGA)

Over 100 patients have been reported with L-2-OHGA, which is caused by deficiency of L-2-hydroxyglutaryl-CoA dehydrogenase activity and characterized by accumulation and increased urinary excretion of L-2-hydroxyglutaric acid (L-2-OHG) (Shafeghati et al. 2006; Steenweg et al. 2009, 2010). The biochemical hallmark of this disorder is elevated levels of L-2-hydroxyglutaric acid in urine, CSF and to a lesser extent in plasma. Affected patients have neurologic manifestations, including progressive mental deficiency, variable motor impairment, cerebellar ataxia and a typical MRI profile with striking subcortical leucoencephalopathy and basal ganglia abnormalities, resembling late-onset mitochondrial encephalopathy (Barth et al. 1992, 1998). Lactate and some TCA intermediates are found in CSF and plasma of patients, with signal changes in the basal ganglia (Hoffmann et al. 1995; Barth et al. 1998). Treatment of one patient with riboflavin resulted in improvement in cognitive and motor performance and a decrease in the urinary excretion of L-2OHG (Yilmaz 2009). In addition, studies in the rat have shown that L-2-OHGA inhibits mitochondrial creatine kinase activity in the cerebellum (da Silva et al. 2003b), suggesting a disturbance of cell energy transfer and buffering.

## Ethylmalonic encephalopathy (EE)

EE is an autosomal recessive disorder caused by mutations in the mitochondrial sulfur dioxygenase encoded by the ETHE1 (Tiranti et al. 2004, 2009). Over 30 patients, mainly of Mediterranean descent, are known. Although the exact role of the gene product is unknown, it seems to be involved in the homeostasis of mitochondrial metabolism. EE is characterized by psychomotor regression and generalized hypotonia, later evolving into spastic tetraparesis, dystonia, and eventually global neurological failure, as well as chronic diarrhea and diffuse petechiae. Magnetic resonance imaging (MRI) examination shows the presence of symmetrical and asymmetrical “patchy” lesions, distributed in the deep grey structures of the brain, including the brain stem, thalamus, and corpus striatum.(Grosso et al. 2002; Zafeiriou et al. 2007). Laboratory values include increased ethylmalonic acid, persistent lactic acidemia with increased plasma alanine, and reduced activity of respiratory complexes IV and II in skeletal muscle (Lehnert and Ruitenbeek 1993; Zafeiriou et al. 2007). One study with Magnetic ressonance spectroscopy revealed that brain energy metabolism is probably impaired in this disorder (Grosso et al. 2004). Other investigators observed moderate biochemical and/or clinical improvement in patients with EE treated with riboflavin (Yoon et al. 2001;

Zafeiriou et al. 2007), further supporting the view that respiratory chain function is compromised in EE. It was postulated by these authors that the biochemical features of EE that are indicative of mitochondrial impairment may result from the accumulated metabolites or due to alterations of one or more nuclear-encoded subunits of the respiratory chain complexes. Experimental evidence support a condition of mitochondrial dysfunction caused by ethylmalonic acid that inhibits the electron transport chain and mitochondrial creatine kinase activity in humans skeletal muscle and in rat cerebral cortex (Leipnitz et al. 2003; Barschak et al. 2006).

## Concluding remarks: Implications for treatment of OAs

Mitochondrial dysfunction, probably caused by the toxic accumulation of acids or coenzyme A ester derivatives, may underlie at least in part the chronic and especially the acute neurological symptoms and abnormalities observed in some OAs. However, the available data in humans and experimental models revealing compromised bioenergetics must be taken with caution in order to better evaluate and validate the relevance of this pathomechanism, relatively to others such as oxidative stress and excitotoxicity. Nevertheless, targeting the mitochondrion with suitable drugs, first in animal models of OAs, and in the future as potential therapies for humans affected by these diseases may become an important focus for future therapeutic approaches. We expect that this review article may encourage additional investigations in this area.

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