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Review

Pathogenesis of brain damage in glutaric acidemia type I: Lessons from the genetic mice model

Moacir Wajner[a,b,c,*], Alexandre Umpierrez Amaral[a,b,d], Guilhian Leipnitz[a,b], Bianca Seminotti[a,b]

[a] Programa de Pós-Graduaçao em Ciências Biológicas: Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

[b] Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Rua Ramiro Barcelos, 2600, Prédio, 21111, Porto Alegre, RS, 90035-003, Brazil

[c] Serviço de Genética Médica, Hospital de Clínicas de Porto Alegre, Rua Ramiro Barcelos, 2350, Porto Alegre, RS, 90035-007, Brazil

[d] Departamento de Ciências Biológicas, Universidade Regional Integrada do Alto Uruguai e das Missões, Avenida Sete de Setembro, 1621, Erechim, RS, 99709-910, Brazil

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ABSTRACT

Glutaric acidemia type I (GA I) is an inherited neurometabolic disease caused by deficient activity of the mitochondrial enzyme glutaryl-CoA dehydrogenase (GCDH), resulting in predominant accumulation of glutaric and 3-hydroxyglutaric acids derived from lysine (Lys), hydroxylysine, and tryptophan catabolism. GA I patients usually present progressive cortical leukodystrophy and frequently develop acute striatal degeneration during encephalopathic crises during the first three years of life. The pathophysiology of the neurodegeneration observed in GA I is still partly known, although the development of the genetic mice model of GA I (*Gcdh*$^{-/-}$) has contributed to clarify potential underlying mechanisms involved in brain damage in this disease. In this review we will summarize the knowledge acquired from studies using this animal model indicating that disruption of redox homeostasis, glutamatergic neurotransmission and bioenergetics, as well as vascular alterations, blood-brain barrier breakage and altered myelination underlie the cortical and striatum abnormalities and white matter changes observed in GA I patients. Elucidation of these pathomechanisms potentially offers new standpoints for the development of novel therapeutic strategies for this disease.

1. Glutaric acidemia type I (GA I)

Glutaric acidemia type I (GA I; OMIM # 231,670) is an inherited autosomal recessive neurometabolic disease caused by mutations in the gene encoding the mitochondrial enzyme glutaryl-CoA dehydrogenase (GCDH; EC 1.3.99.7) of the catabolic pathway of the amino acids lysine (Lys), hydroxylysine and tryptophan (Goodman et al., 1977). The GCDH gene is located on human chromosome 19p13.2, spans about 7 kb and comprises 11 exons and 10 introns (Goodman et al., 1977; Shadmehri et al., 2019). GCDH catalyzes the dehydrogenation of glutaryl-CoA to glutaconyl-CoA and its decarboxylation to crotonyl-CoA. Deficiency of GCDH activity leads to predominant tissue accumulation and high urinary excretion of glutaric acid (GA) and 3-hydroxyglutaric acid (3−OHGA) (Goodman et al., 2001).

The estimated worldwide frequency of GA I is between 1:30,000 to 1:100,000 newborns, being one of the most prevalent organic acidurias (Goodman et al., 2001; Lindner et al., 2004; Wajner et al., 2009). Affected patients present at birth with macrocephaly and frontotemporal cortical atrophy. Encephalopathic crises manifested by convulsions generally triggered by fever, infections or prolonged fasting occur in two third of affected patients during the first 3 years of life. These episodes are associated with severe striatum degeneration and followed by dystonia, dyskinesia, muscle stiffness and general developmental deterioration. GA I is considered a cerebral organic aciduria because

Abbreviations: Akt, protein kinase B; BBB, blood-brain barrier; CK, creatine kinase; DCFH, 2′,7′-dichlorofluorescin; Erk 1/2, extracellular signal-regulated kinase; GA, glutaric acid; GA I, glutaric acidemia type I; GCDH, glutaryl-CoA dehydrogenase; *Gcdh*−/−, glutaryl-CoA dehydrogenase deficient; GR, glutathione reductase; GSH, reduced glutathione; GPx, glutathione peroxidase; GST, glutathione S-transferase; 3−OHGA, 3-hydroxyglutaric acid; IκBα, NF-kappa-B inhibitor alpha; Keap 1, Kelch-like ECH-associated protein 1; Lys, lysine; MDA, malondialdehyde; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NMDA, *N*-methyl-D-aspartate; Nrf2, nuclear factor (erythroid-derived 2)-like 2; OXPHOS, oxidative phosphorylation; QA, quinolinic acid; ROS, reactive oxygen species; SOD, superoxide dismutase; SOD2, superoxide dismutase-2; VEGF, vascular endothelial growth factor; WT, wild type

* Corresponding author at: Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Rua Ramiro Barcelos, 2600, Prédio 21111, 90035-003, Porto Alegre, RS, Brazil.

E-mail address: mwajner@ufrgs.br (M. Wajner).

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symptomatology is mainly or exclusively neurologic (Goodman et al., 1977; Neumaier-Probst et al., 2004; Kölker et al., 2006; Boy et al., 2017a). Insidious or late-onset (clinical manifestations after 6 years of age) with striatum abnormalities and progressive cortical leukodystrophy associated with white matter changes are also found (Neumaier-Probst et al., 2004; Funk et al., 2005; Strauss et al., 2007; Harting et al., 2009; Garbade et al., 2014). However, the pathomechanisms responsible for the neurodegeneration in GA I are still not completely elucidated (Goodman et al., 2001; Jafari et el., 2011).

Diagnosis is mostly performed by elevated levels of GA and 3 − OHGA in urine, as well as by glutarylcarnitine (C5DC) in blood of patients and confirmed by detection of deficient activity of GCDH activity in fibroblasts or leukocytes. The prognosis of GA I depends on early diagnosis and treatment based on restricted intake of Lys/protein, as well as by supplementation of L-carnitine and in some cases arginine (Goodman et al., 2001; Kölker et al., 2002; Boy et al., 2017a; Guerreiro et al., 2018).

2. Genetic mice model of GA I

A gene targeting vector was created by replacing exons 1–7 of the Gcdh gene with the nlacF and NEO genes. The final vector contained ~8.5 kb of homologous DNA 5′ of the nlacF gene and 700 bp of homologous DNA 3′ of the NEO cassette. Transfected J1 ES cells derived from 129 × 1/SvJ mice were selected and screened for homologous insertion by PCR analysis using two different pairs of primers. The PCR product was confirmed by restriction analysis. Correctly targeted ES cells were injected into C57Bl/6 J blastocysts, which were transferred to the uteri of pseudopregnant females for gestation. Chimeric male animals were crossed to C57Bl/6 J females and the progeny were screened for the presence of the targeted Gcdh mutation by PCR (Koeller et al., 2002).

GCDH deficient (*Gcdh*$^{-/-}$) mice present high levels of GA and 3 − OHGA, similar to GA I patients. They also manifest mild motor deficit and spongiform myelinopathy, but do not present striatum degeneration associated with neuronal loss and astrogliosis that are characteristic findings of the human condition. An improvement of this model was achieved by exposing *Gcdh*$^{-/-}$ mice to a high Lys or protein overload (Zinnanti et al., 2006, 2007). In this model, high Lys diet for 72 h induced vasogenic edema, blood-brain barrier (BBB) breakdown, neuronal loss, hemorrhage within the striatum, as well as paralysis, seizures and death in 4-week-old *Gcdh*$^{-/-}$ mice. Older animals (8-week-old *Gcdh*$^{-/-}$ mice) on high Lys chow for 6 weeks survived but developed more intensive white matter lesions, reactive astrocytes and striatum neuronal loss (Zinnanti et al., 2006). This *Gcdh*$^{-/-}$ mouse model exposed to Lys overload has been since preferentially used as an appropriate GA I animal model to study the neuropathology of this disorder.

Noteworthy, previous *in vivo* animal models of GA I were developed by administration of GA or 3 − OHGA by subcutaneous, intraperitoneal, intrastriatal or intracerebroventricular injection to wild rats with normal GCDH activity. *In vitro* studies were also carried out by supplementing GA or 3 − OHGA to brain fresh tissue, neural cells or organelles. These models allowed to separately study the specific effects of these accumulating organic acids on central nervous system and delivered important data on the neuropathology of GA I. However, since knock out animal models of diseases, including the *Gcdh*$^{-/-}$ mice, better mimic human pathologic conditions, they have been increasingly utilized for pathophysiologic investigation.

Therefore, the purpose of the present review was to summarize the major pathomechanisms of striatum and cerebral cortex obtained by experimental studies using the *Gcdh*$^{-/-}$ mice.

3. Oxidative stress and neuroinflammation in *Gcdh*$^{-/-}$ mice

Growing evidence indicates that oxidative stress is induced in *Gcdh*$^{-/-}$ mice, particularly when exposed to Lys overload. In this context, although redox homeostasis was unaltered in cerebral cortex and striatum from 30-day-old *Gcdh*$^{-/-}$ mice fed a normal chow, a single intraperitoneal injection of Lys that leads to higher brain GA and 3 − OHGA concentrations, mimicking an acute encephalopathic crisis, provoked marked lipid peroxidation and reactive oxygen species (ROS) production, besides increasing superoxide dismutase (SOD) and glutathione reductase (GR) activities and decreasing reduced glutathione (GSH) levels and glutathione peroxidase (GPx) activity in striatum of *Gcdh*$^{-/-}$ animals (Seminotti et al., 2012). Increased lipid peroxidation and SOD activity and a decrease of GSH levels were also observed in the cerebral cortex, besides mild increases of SOD activity and reactive species generation in the hippocampus of *Gcdh*$^{-/-}$ mice. Furthermore, no alteration of these oxidative stress parameters occurred in heart and liver of *Gcdh*$^{-/-}$ animals pointing to a higher vulnerability of brain to this insult (Seminotti et al., 2012).

In line with these findings, acute Lys intraperitoneal injection also provoked disruption of redox homeostasis in younger animals (15-day-old *Gcdh*$^{-/-}$ mice), as evidenced by decreased GSH concentrations in brain and of sulfhydryl content in liver, apart from increased carbonyl formation and 2′,7′-dichlorofluorescin (DCFH) oxidation and decreased activities of antioxidant enzymes in brain and liver (Seminotti et al., 2014).

Furthermore, chronic Lys overload achieved by high Lys (2.8% and 4.7%) chow, mimicking the human insidious and progressive condition, elicited noticeable oxidative stress associated with histopathological findings in striatum and cerebral cortex, but not in hippocampus, liver and heart of 30-day-old *Gcdh*$^{-/-}$ mice (Seminotti et al., 2013). Under this dietary regime, it was observed an increase of malondialdehyde (MDA) levels and DCFH oxidation, indicating lipid peroxidation and increased ROS generation, respectively, in striatum and cerebral cortex of the mutant mice. Antioxidant defenses were also altered in these animals, as seen by reduction of GSH levels and changes of antioxidant enzyme activities in these cerebral tissues. In the same study histopathological analysis showed increased expression of oxidative stress markers, despite the absence of significant anatomic brain damage. Another interesting observation was that oxidative stress induction in cerebral cortex and striatum was more accentuated in symptomatic, as compared to asymptomatic *Gcdh*$^{-/-}$ mice exposed to a 4.7% high Lys diet. Table 1 summarizes the data obtained in tissues from *Gcdh*$^{-/-}$ mice, strongly indicating that oxidative stress may represent a relevant pathomechanism of brain damage in this genetic mouse model of GA I.

Considering that neuroinflammation is commonly associated with oxidative stress and thought to have an important role in various neurologic diseases (Dasuri et al., 2013; Freeman et al., 2016), the effects of quinolinic acid (QA), whose synthesis is activated in the kynurenine pathway during inflammatory processes, on redox homeostasis was also evaluated in brain of *Gcdh*$^{-/-}$ mice under Lys overload. Overall it was shown that a single intrastriatal injection of QA to 30-day-old *Gcdh*$^{-/-}$ mice exposed to a high Lys diet provoked marked disruption of redox homeostasis (Seminotti et al., 2016). QA induced lipid and protein oxidative damage in striatum of *Gcdh-/-* mice, possibly secondarily to the increased generation of reactive oxygen and nitrogen species that were activated. It also decreased GSH levels and altered the activities of GPx, superoxide dismutase-2 (SOD_2) and glutathione S-transferase (GST). Moreover, QA augmented the levels of Akt and Erk 1/2 phosphorylation that participate in various signaling pathways, including Nrf2 and NF-kB translocation. The levels of these transcription factors (Nrf2 and NF-kB) were increased in nucleus, while their cytosolic inhibitory proteins Keap1 and IκBα were decreased. Noteworthy, both signaling pathways (NRF2/keap1 and NFKB/ikba) are involved in oxidative stress, antioxidant response and inflammation. Otherwise, QA provoked extensive vacuolation and edema in *Gcdh-/-* mice striatum. A further study showed that QA enhanced CD3 staining and the number of YNO2 positive cells in these animals, implying T lymphocyte infiltration and nitrosative stress, respectively

Table 1
Disruption of redox homeostasis in Lys-treated *Gcdh*$^{-/-}$ mice.

Animal age	Lys overload	Tissue	Oxidative stress parameters
Fifteen-day-old *Gcdh*$^{-/-}$	I.P. Lys injection (8 μmol/g)	Brain Liver	↓ GSH levels, ↑ carbonyl formation, ↑ DCFH oxidation, ↓ GPx, ↓SOD, ↓CAT, ↓GR ↓ sulfhydryl content, ↑ carbonyl formation, ↑ DCFH oxidation
Thirty-day-old *Gcdh*$^{-/-}$	I.P. Lys injection (8 μmol/g)	Striatum Cerebral cortex Hippocampus Liver, heart	↑ MDA levels, ↑ DCFH oxidation, ↓ GSH levels, ↑ SOD, ↑ GR, ↓ GPx ↑ MDA, ↑ SOD, ↓ GSH levels ↑ SOD, ↑ DCFH oxidation no alterations
Thirty-day-old *Gcdh*$^{-/-}$	Lys enriched chow (2.8% or 4.7%) for 60 hours	Striatum Cerebral cortex Hippocampus, liver, heart	↑ MDA, ↓ GSH levels, ↑ YNO2, ↑ iNOS ↑ MDA, ↑ DCFH oxidation, ↓ GSH levels, ↑ SOD, ↑ CAT no alterations
Thirty-day-old *Gcdh*$^{-/-}$	Lys enriched chow (4.7%) plus intrastriatal QA injection	Striatum	↑ MDA levels, ↑ DCFH oxidation, ↑ Nitrate and nitrite levels, ↓ GSH levels, ↓ sulfhydryl content, ↑ GPx, ↑ SOD2, ↑ GST, ↑ Nrf2, ↑ NF-κB, ↑ Akt, ↑ Erk 1/2 phosphorilation, ↓ Keap, ↓ IκBα

(Seminotti et al., 2012, 2013, 2014, 2016). Akt - protein kinase B; CAT - catalase; DCFH - 2′,7′-dichlorofluorescin; Erk ½ - extracellular signal-regulated kinase; GPx - glutathione peroxidase; GR - glutathione reductase; GSH - reduced glutathione; GST - glutathione S-transferase; IκBα - NF-kappa-B inhibitor alpha; I.P. - intraperitoneal; iNOS - inducible nitric oxide synthetase; Keap 1 - Kelch-like ECH-associated protein 1; MDA – malondialdehyde; NF-κB - nuclear factor kappa-light-chain-enhancer of activated B cells; Nrf2 - nuclear factor (erythroid-derived 2)-like 2; SOD - superoxide dismutase; YNO2 - nitrotyrosine.

(Amaral et al., 2018). Synergistic toxic effects of QA and GA in neuronal cultures, co-cultures and mixed cultures from WT rat cerebral cortex and striatum has been also recently described, strengthening a synergistic role of QA and GA in GA I neurodegeneration (Pierozan et al., 2018; Kotlar et al., 2019). Taken together, these observations support the hypothesis that QA may potentially contribute to GA I neuropathogenesis, as originally proposed (Varadkar and Surtees, 2004). However, this assumption should be interpreted with caution since to the best of our knowledge there is no report showing increased brain levels of QA or its substrate tryptophan in brain of *Gcdh*$^{-/-}$ mice or glutaric acidemic patients even during infectious that are associated with inflammation.

4. Glutamatergic and GABAergic neurotransmission impairment in *Gcdh*$^{-/-}$ mice

Disturbances of the glutamatergic and GABAergic systems have been demonstrated in brain of *Gcdh*$^{-/-}$ mice, indicating that excitotoxicity may be involved in the neuropathology of GA I. As far as the glutamatergic system is concerned, it was verified that glutamate binding to Na^+-dependent transporters was increased in the striatum of adult *Gcdh*$^{-/-}$ mice, and this was hypothetically attributed to either a higher expression or affinity of glutamate transporters in order to uptake excessive glutamate from the synaptic cleft (Busanello et al., 2014). Furthermore, the addition of exogenous GA to cerebral cortex and striatum of these knockout mice provoked a significant decrease of [^{3}H] glutamate uptake and Na^+-dependent glutamate binding in these brain structures, suggesting a competition between GA and glutamate for high-affinity glutamate transporters because of their chemical structural similarity.

Other studies revealed marked elevations of mRNA levels of the glutamate NMDA receptor subtypes NR2A and NR2B in striatum and of the levels of GluR2 and GluR6 subtypes in cerebral cortex of 7-day-old *Gcdh*$^{-/-}$ mice. Increased mRNA expression of the NMDA subunits NR1, NR2A and NR2B in cerebral cortex and of NR2A and NR2B in striatum of 30-day-old *Gcdh*$^{-/-}$ mice was also observed. Finally, overexpression of all glutamate ionotropic receptors was demonstrated in cerebral cortex and striatum of adult (60-day-old) *Gcdh*$^{-/-}$ mice (Lagranha et al., 2014). These results support the findings showing increased glutamate binding to Na^+-dependent transporters (Busanello et al., 2014). Higher expression of the glutamate transporter subunits GLAST and GLT1 was also seen in cerebral cortex and striatum of adult *Gcdh*$^{-/-}$ mice, whereas in infant mice only GLAST mRNA levels were augmented in striatum. Furthermore, Lys overload was able to further increase the expression of these glutamate receptors and transporters, implying a role for GA and 3−OHGA.

Another study corroborated these findings showing that *Gcdh*$^{-/-}$ mice fed a high Lys diet present significant increase of NR2B, NR1 and NR2A mRNA levels in the striatum (Rodrigues et al., 2015). Taken together, these data indicate that accumulation of GA and 3−OHGA originated from Lys induce overexpression of glutamate receptors and transporters therefore potentially facilitating excitotoxicity These data obtained in the knockout genetic model of GA I are in accordance with the observations that GA and 3−OHGA disturb various parameters of the glutamatergic system in cerebral cortex and striatum of rats (Lima et al., 1998; Porciúncula et al., 2000, 2004; Kölker et al., 2002; Frizzo et al., 2004; Rosa et al., 2004, 2007; Wajner et al., 2004; Dalcin et al., 2007).

On the other hand, it was also demonstrated that the GABAergic system is also impaired in GA I, as indicated by the reduced concentrations of the inhibitory neuromodulator GABA found in *postmortem* brain tissue from *Gcdh*$^{-/-}$ mice and patients with GA I (Leibel et al., 1980; Funk et al., 2005; Zinnanti et al., 2007). Lower GABA levels were attributed to inhibition of glutamate decarboxylase activity by GA and 3−OHGA (Stokke et al., 1976). Severe changes of electrophysiological findings observed in the *Gcdh*$^{-/-}$ mice also suggest disruption of GABAergic neurotransmission system in GA I (Vendramin Pasquetti et al., 2017).

It is therefore conceivable that disturbance of glutamatergic and GABAergic systems may induce excitotoxicity and be associated with the reduced number of striatal medium spiny neurons found in *postmortem* brain of *Gcdh*$^{-/-}$ mice and patients with GA I (Goodman et al., 1977; Funk et al., 2005; Zinnanti et al., 2007). Of note, these neurons are highly vulnerable to excitotoxicity (Calabresi et al., 1998). In summary, excitotoxicity may be possibly considered an important pathogenic mechanism in GA I neurodegeneration.

5. Bioenergetics dysfunction in *Gcdh*$^{-/-}$ mice

Mitochondria are essential for cellular functioning and survival. This organelle is mainly responsible for energy production and transfer, crucial for cellular redox and calcium homeostasis, and have a central role in apoptosis (Nicholls and Ferguson, 2013). Disturbed mitochondrial functions have been associated with encephalopathy of common neurological disorders (Farshbaf and Ghaedi, 2017; Guo et al., 2017; Tefera and Borges, 2017; Angelova and Abramov, 2018; Zhou et al., 2018), as well as in brain injury of GA I (Strauss and Morton, 2003; Kölker et al., 2004; Wajner and Goodman, 2011).

As far as GA I is concerned, swollen and disintegrating brain mitochondria, associated with depletion of ATP, phosphocreatine,

coenzyme A, α-ketoglutarate, glutamate, glutamine and GABA were observed in young *Gcdh*$^{-/-}$ mice fed a high Lys chow (Zinnanti et al., 2007). Decreases of CK, Na^+, K^+ - ATPase and citric acid cycle activities in brain of developing *Gcdh*$^{-/-}$ mice submitted to Lys overload were also observed (Amaral et al., 2012a, 2012b, 2015; Sauer et al., 2015). Moreover, intrastriatal injection of QA caused a marked inhibition of complex IV and CK activities in striatum of Lys-treated *Gcdh*$^{-/-}$ mice (Seminotti et al., 2016). It was also observed a reduced efflux of succinate from *Gcdh*$^{-/-}$ astrocytes potentially disturbing the pleiotropic flux of substrates into neural cells (Lamp et al., 2011).

Glutarylation of mitochondrial enzymes such as glutamate dehydrogenase and citric acid cycle enzymes were shown to affect their stability and activity in glial cells of *Gcdh*$^{-/-}$ mice (Schmiesing et al., 2018). These functionally disturbed glutarylated proteins were associated with elevated levels of GA and 3−OHGA. It was also proposed that this post-translational modification impair the anaplerotic transfer of citric acid cycle intermediates and glutamine from astrocytes to neurons therefore affecting ATP and neurotransmitter synthesis.

Taken together, the data obtained from *Gcdh*$^{-/-}$ mice support the hypothesis that mitochondrial dysfunction is also a contributing factor to the neuropathology of GA I. This is in line with the large number of *in vitro* and *in vivo* studies performed in rats with normal GCDH activity demonstrating that GA and 3−OHGA provoke mitochondrial disruption, by inhibiting the respiratory chain, CK and Na^+, K^+ - ATPase activities (Silva et al., 2000; Kölker et al., 2002; Ferreira et al., 2005a,b, 2007a,b; Latini et al., 2005; Olivera et al., 2008), suggesting that disturbance of bioenergetics in GA I is caused by the major accumulating metabolites.

6. Astrogliosis in *Gcdh*$^{-/-}$ mice

Astrogliosis is a condition that may be triggered by brain insults and characterized by significant cellular and functional alterations in glial cells, particularly astrocytes (Zhang et al., 2010). Since gliosis has been observed in *post-mortem* brains of GA I patients (Goodman et al., 1977, 2001; Funk et al., 2005), different studies investigated the involvement of glial reactivity in the pathophysiology of brain damage in *Gcdh*$^{-/-}$ mice. In this scenario, a pioneer study revealed a high degree of astrogliosis with increased number of reactive astrocytes adjacent to blood vessels with end-foot thickening in cerebral cortex of *Gcdh*$^{-/-}$ mice fed a high Lys chow (Zinnanti et al., 2006). A further investigation has shown that exposition of cultured astrocytes of striatum of *Gcdh*$^{-/-}$ mice to high Lys and GA concentrations induce their proliferation as well as oxidative stress that can be prevented by the antioxidants melatonin an alpha-tocopherol (Olivera-Bravo et al., 2015). Moreover, it was seen that these *Gcdh*$^{-/-}$ striatum astrocytes exposed to Lys synthetize and release GA and 3−OHGA in the culture medium, become hyperactive and provoke cortical neuronal death that was prevented by melatonin and alpha-tocopherol. These findings suggest that hyperactive astrocytes became neurotoxic probably through increased GA and 3−OHGA production and are able to kill striatal and cortical neurons by an oxidative stress-dependent mechanism (Olivera-Bravo et al., 2015).

A very recent report using *Gcdh*$^{-/-}$ 3D organotypic brain cell cultures demonstrated changes in astrocytes morphology, including shorter, thicker, and wavy astrocytic fibers in *Gcdh*$^{-/-}$ mice. It was also seen that exposure of these cultured cells to Lys increased the morphological alterations observed in *Gcdh*$^{-/-}$ brain cells (Cudré-Cung et al., 2019). More important, these findings are similar to the histopathological and immunohistochemical findings observed in autopsied brain of GA I patients, showing reactive and hypertrophic astrocytes, as well as microglial activation especially in basal ganglia, which is an indicative of chronic astrogliosis (Goodman et al., 1977, 2001; Funk et al., 2005).

7. Vascular alterations and disturbance of blood-brain barrier (BBB) permeability in *Gcdh*$^{-/-}$ mice

Patients affected by GA I present cerebral vascular changes with intracranial and intradural hemorrhages, as well as chronic subdural effusions often associated with acute striatal degeneration mostly occurring during episodes of metabolic decompensation (Woelfle et al., 1996; Strauss et al., 2007). In this context, data obtained with microarray analysis revealed upregulation of the genes of vascular endothelial growth factor (VEGF) and angiogenin in brain of *Gcdh*-/- mice (Mühlhausen et al., 2004). It was also verified that 3−OHGA inhibits *in vitro* the migration of endothelial cells, impairs the integrity of capillaries and increases vascular permeability in human dermal microvascular endothelial cells (Mühlhausen et al., 2004, 2006). In addition, Zinnanti et al. (2006) demonstrated that 4-week-old *Gcdh*$^{-/-}$ mice fed a high Lys chow showed extravasation of Evans blue in striatum implying BBB disruption in these animals. The same investigation also showed that high concentrations of GA and 3−OHGA increase the permeability of microvessels prepared from rat striatum and cerebral cortex (Zinnanti et al., 2006). Taken together, these experimental findings indicate that GA and 3−OHGA induce vascular derangements and BBB breakdown.

Another study evidenced that *Gcdh*$^{-/-}$ mice fed a high protein chow have neuronal swelling and vacuole formation associated with cortical and striatal capillary occlusion and ischemia (Zinnanti et al., 2014). When these mice were fed either a normal or a high Lys diet there was an increase in VEGF content in both cerebral cortex and striatum leading to a decrease in the staining of the tight-junction protein occludin located in the BBB, that was proposed to be associated with BBB breakdown. Of note, VEGF is a growth factor that has a central role in occludin removal from BBB (Murakami et al., 2009). Subarachnoidal, subdural and intraventricular hemorrhages and BBB breakdown were also observed in *Gcdh*$^{-/-}$ animals receiving a high Lys chow (Sauer et al., 2015). Altogether, these data show solid evidence of vascular alterations and potential underlying mechanisms, as well as BBB disruption in the genetic mouse model of GA I subjected to Lys overload that may possibly explain the cerebral vascular changes observed in GA I patients. Since these vascular abnormalities observed in *Gcdh*$^{-/-}$ animals were achieved with Lys overload that leads to brain accumulation of GA and 3−OHGA (Zinnanti et al., 2006), it may be presumed that these accumulating organic acids may underlie these changes. This is in line with what happens in the human condition in which intracranial and intradural hemorrhages, as well as chronic subdural effusions are frequently concomitant with the episodes of metabolic decompensation biochemically characterized by dramatic increases of the accumulated organic acids.

8. White matter injury in *Gcdh*$^{-/-}$ mice

Diffuse white matter abnormalities with hypomyelination (delayed myelination) are commonly observed in GA I patients, especially in those affected by the late-onset or insidious forms that may not be accompanied by encephalopathic crises (Oguz et al., 2005; Strauss et al., 2007; Harting et al., 2009; Boy et al., 2017b). *Gcdh*$^{-/-}$ mice also present white matter changes. It was demonstrated that adult *Gcdh*$^{-/-}$ mice present moderate vacuolation of the white matter in the cerebral cortex and hippocampus (Koeller et al., 2002; Zinnanti et al., 2006; Sauer et al., 2015), that were accentuated and could be visualized in the striatum when these animals were exposed to a chronic high Lys chow indicating a role for GA and 3−OHGA in this process (Zinnanti et al., 2006; Amaral et al., 2015).

Since disruption of myelination does not seem to correlate with the acute crises of encephalopathy associated with striatum degeneration and motor disabilities, differential pathomechanisms may be acting in the myelinopathy and in the acute striatal damage with death of medium spiny neurons that frequently occur in GA I. In this context,

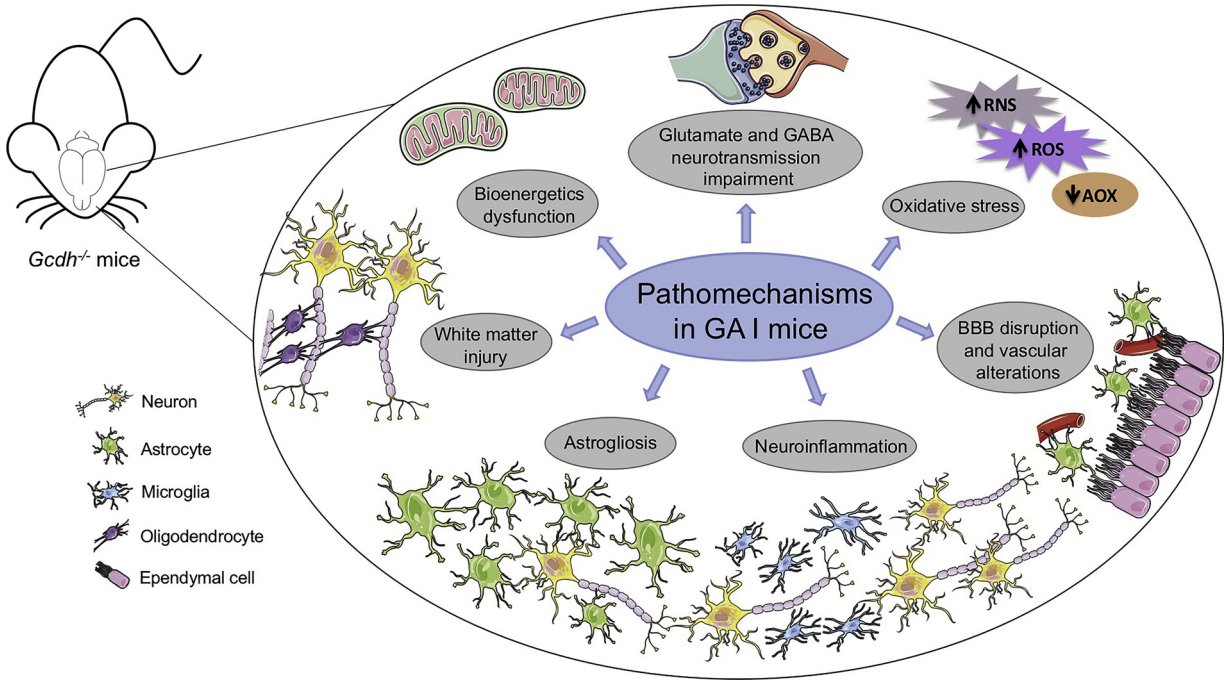

Fig. 1. Schematic representation of the mechanisms involved in the pathogenesis of the brain damage in the genetic mouse model of glutaric acidemia type I (GA I). Oxidative stress, neuroinflammation, mitochondrial bioenergetics dysfunction, glutamatergic and GABAergic neurotransmission impairment, astrogliosis, vascular abnormalities, disturbance of blood-brain barrier permeability and white matter injury were observed in brain of glutaryl-CoA dehydrogenase deficient (*Gcdh*$^{-/-}$) mice.

Olivera-Bravo and collaborators (2019) verified a significant decrease of striatal-myelinated areas and progressive vacuolation of white matter in adult *Gcdh*$^{-/-}$ mice chronically exposed to a moderate high increase of Lys chow (2.8% Lys) for up to 60 days that is thought to provoke sustained high levels of GA I accumulating metabolites. The same authors demonstrated that GRP78/BiP immunoreactivity, a marker of endoplasmic reticulum stress, was significantly increased in oligodendrocytes from *Gcdh*$^{-/-}$ mice fed with this chow, albeit neuronal density was not disturbed in brain of these animals (Olivera-Bravo et al., 2019). These data suggest that the hypomyelination observed in the striatum of *Gcdh*$^{-/-}$ mice under high Lys chow is associated with endoplasmic reticulum stress and not to neuronal death, supporting distinct mechanisms for these processes. In line with these observations, a recent work showed decreased expression of galactocerebrosidase and myelin basic protein in 3D organotypic cell cultures prepared from *Gcdh*$^{-/-}$ brain that was accentuated by Lys exposure and may result from a reduction in the number of oligodendrocytes and/or a delayed myelination in the brain of these animals (Cudré-Cung et al., 2019), similarly to what is found in cerebral magnetic resonance imaging of GA I patients (Harting et al., 2009, 2015; Boy et al., 2017b). It was also demonstrated decreased density of neuronal fibers in axonal and dendrites of *Gcdh*$^{-/-}$ brain cell cultures that was further reduced by Lys exposure. In contrast, the number of neurons was not changed in the *Gcdh*$^{-/-}$ cell culture (Cudré-Cung et al., 2019).

9. Concluding remarks

The development of the genetic mouse model of GA I (*Gcdh*$^{-/-}$) has been proven to be very valuable to elucidate the pathogenesis of this disease in the last few years especially in what concerns to the characteristic neurodegeneration, *i.e.* acute striatal degeneration and progressive cortical damage evolving to leukodystrophy. So, an increasing number of studies have shown that disruption of redox and bioenergetics homeostasis, neuroinflammation, disturbance of the glutamatergic and GABAergic neurotransmission systems, vascular abnormalities, blood brain barrier breakage and myelinopathy associated with endoplasmatic reticulum stress play an important role in the striatum and cerebral cortex damage in *Gcdh*$^{-/-}$ mice. Many of these works also observed that these alterations were accentuated in knock out animals receiving high Lys chow or acute Lys administration, implying toxicity of the accumulating organic acids GA and 3−OHGA that are generated in the brain by these treatments (Zinnanti et al., 2006; Seminotti et al., 2012, 2013, 2014; Olivera-Bravo et al., 2015, 2019). Fig. 1 summarizes potential pathomechanisms underlying the neurological symptoms and brain abnormalities observed in GA I. Although it is difficult to establish the relevance of each mechanism, the available data in the literature obtained from patients and animal models of GA I indicate that oxidative stress associated with neuroinflammation and excitotoxicity induced by the major accumulating organic acids (GA and 3−OHGA) play a key role in GA I pathophysiology. In this particular, recent findings obtained in blood and urine of patients with GA I support oxidative stress associated with inflammation in the pathogenesis of this disease (Guerreiro et al., 2018). Finally, investigation of the role of mitochondrial targeted antioxidants or compounds that increase mitochondrial biogenesis and dynamics, such as resveratrol and bezafibrate, firstly in *Gcdh-/-* mice tissues and cells from patients affected by GA I, may represent in the future novel therapies aiming to reduce reactive species levels and improve cellular respiration in this disease. In this particular, beneficial effects of these compounds have been demonstrated in animal models and fibroblasts from patients with fatty acid oxidation and respiratory chain defects (Bonnefont et al., 2009; Aires et al., 2014; Ørngreen et al., 2014; Djouadi and Bastin, 2019). We expect that this review article may encourage additional investigations in this area.

Conflict of interest

The authors declare no conflict of interest.

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