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Pathophysiology of maple syrup urine disease: Focus on the neurotoxic role of the accumulated branched-chain amino acids and branched-chain α-keto acids

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ABSTRACT

Maple syrup urine disease (MSUD) is an autosomal recessive neurometabolic disorder caused by severe deficiency of branched-chain α-keto acid dehydrogenase complex activity, which catalyzes the oxidative decarboxylation of the branched-chain α-keto acids (BCKA). The metabolic blockage results in tissue accumulation and high urinary excretion of the branched-chain amino acids (BCAA) leucine, isoleucine and valine, as well as alloisoleucine, and their respective BCKA α-ketoisocaproic (α-KIC), α-ketoisovaleric and α-keto-β-methylvaleric acids. Affected patients usually manifest acute episodes of encephalopathy associated with seizures, coma and life-threatening cerebral edema in the first weeks of life, which is followed by progressive neurological deterioration with motor delay, ataxia, intellectual disability and psychiatric symptoms. The pathophysiology of the brain damage in MSUD has been mainly focused on brain amino acid imbalance leading to deficient cerebral protein and neurotransmitter synthesis. However, the acute episodes of severe neurological symptoms accompanied by large increases of BCKA/BCAA levels suggest neurotoxic actions of these compounds. In this particular, mounting evidence from humans and animal models support an important role of particularly leucine and α-KIC on the pathogenesis of the brain injury in MSUD. In this review we will present the current knowledge of the major mechanisms presumably involved in MSUD neuropathology and highlight the neurotoxic properties of the BCAA and BCKA, disturbing brain bioenergetics and redox homeostasis, besides inducing neuroinflammation. We suggest that these pathomechanisms may contribute to the neurological sequelae of MSUD patients and hopefully allow the design of novel therapeutic strategies, including antioxidant and bioenergetics stimulating drugs targeting the mitochondria.

1. Introduction

Maple syrup urine disease (MSUD) or branched-chain ketoaciduria (MIM 248600), first described by Menkes and collaborators (1954), is a rare inherited metabolic disorder caused by a severe deficiency in the activity of the branched-chain α-keto acid dehydrogenase complex (BCKDH; EC 1.2.4.4) (Chuang et al., 2019). It is biochemically characterized by large accumulation of the branched-chain amino acids (BCAA) leucine, isoleucine, and valine, as well as the corresponding branched-chain α-keto acids (BCKA) α-ketoisocaproic (α-KIC), α-ketoisovaleric (α-KIV) and α-keto-β-methylvaleric (α-KMV) acids (Fig. 1) (Chuang et al., 2019).

The prevalence of MSUD in the general population is approximately 1: 150,000 newborns, although it may be much higher in some ethnic groups (Morton et al., 2002; Strauss et al., 2020a). The disease is more severe in patients affected by the classic form, corresponding to approximately 80% of the cases, as compared to the milder forms, i.e. the intermediate, intermittent and thiamine-responsive variants (Chuang et al., 2019; Strauss et al., 2020b). The central nervous system (CNS) is most affected in MSUD. Patients with the classic form commonly present acute episodes of severe vomiting, hypotonia, and

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Abbreviations

BBB	Blood-brain barrier
BCAA	Branched-chain amino acids
BCAT	Branched-chain amino acid transaminase
BCATc	Cytosolic branched-chain amino acid transaminase
BCATm	Mitochondrial branched-chain amino acid transaminase
BCKA	Branched chain α-keto acids
BCKDH	Branched-chain α-keto acid dehydrogenase complex
CAT	Catalase
CNS	Central nervous system
CSF	Cerebrospinal fluid
DBT	Dihydrolipoamide branched-chain transacylase E2
GABA	γ-Aminobutyric acid
Glu	Glutamate
Gln	Glutamine
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Reduced glutathione
8-OHdG	8-Hydroxy-2′-deoxyguanosine
IL-1β	Interleukin-1β
IL-6	Interleukin-6
IL-10	Interleukin-10
INF-γ	Interferon-γ
α-KIC	α-Ketoisocaproic
α-KIV	α-Ketoisovaleric
α-KMV	α-Keto-β-methylvaleric
MDA	Malondyaldehyde
MCT/SLC16A1	Monocarboxylate transporter
MSUD	Maple syrup urine disease
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
LNAA	Large neutral amino acids
LAT1/SLC7A5	Large neutral amino acids membrane carrier system
NMDA	N-methyl-D-aspartate
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
S-AdoMet	S-adenosylmethionine
sICAM-1	Soluble intercellular adhesion molecule-1
sVCAM-1	Soluble vascular cell adhesion molecule-1
SOD	Superoxide dismutase
TAR	total antioxidant reactivity
TNF-α	Tumor necrosis factor α

encephalopathy associated with seizures, coma and life-threatening brain edema during crises of metabolic decompensation, which are accompanied by high increase of BCAA and BCKA concentrations, especially leucine and α-KIC (Chuang et al., 2019).

Diagnosis is based on the detection of increased plasma concentrations of BCAA and of the pathognomonic biomarker alloisoleucine, although high levels of BCKA in urine are also useful to detect this disorder. Diagnostic confirmation can be performed by detection of biallelic pathogenic variants in the dihydrolipoamide branched-chain transacylase E2 (DBT), BCKDHA and BCKDHB genes and by enzymatic activity (Strauss et al., 2020b).

The mainstay of MSUD therapy is based on reducing the accumulation of the toxic BCAA and BCKA by a protein restricted diet, supplemented by BCAA-free amino acid mixtures, containing valine and isoleucine, as well as thiamine for the responsive patients. Liver transplantation has been increasingly used to treat MSUD patients and was shown to significantly reduce mortality and the number of episodes of metabolic decompensation by normalizing the circulating levels of BCAAs without the need of dietary restrictions (Celik et al., 2019; Muelly et al., 2013; Shellmer et al., 2011; Strauss et al., 2020a). Although these treatments significantly increase survival rates, considerable cognitive and psychiatric morbidities remain (Muelly et al., 2013; Strauss et al., 2020a). The mouse model of MSUD suggests that measurement of plasma amino acid is a poor surrogate to evaluate the effect of protein restriction on brain amino acid homeostasis (Vogel et al., 2014). Therefore, it is conceivable that dietary treatment may be insufficient to avoid chronic neurotransmitter disturbances, which may be involved in the long-term neurocognitive dysfunction in MSUD.

In regards to the neuropathological alterations of MSUD patients, cerebral magnetic resonance imaging has shown hypomyelination and cytotoxic intramyelinic sheath edema in the basal ganglia, cerebral cortex, cerebellum, periventricular white matter and brainstem

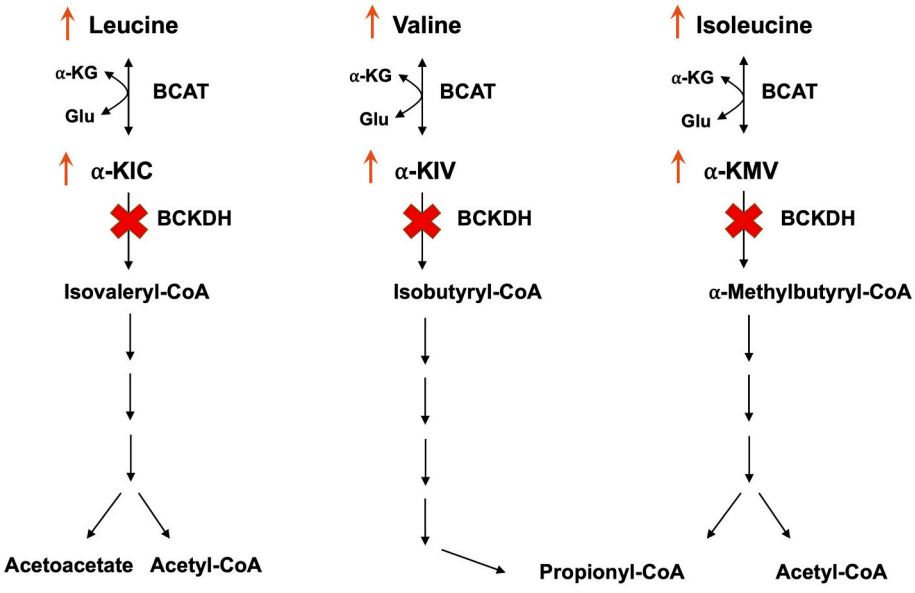

Fig. 1. Catabolic pathways of BCAA and enzymatic defect of MSUD: BCAA and BCKA accumulation. Leucine, valine and isoleucine are first transaminated by BCATc or BCATm forming α-KIC, α-KIV and α-KMV, respectively. MSUD is caused by deficiency of the activity of BCKDH that catalyzes the oxidative decarboxylation of the BCKA, resulting in their accumulation and of their BCAA precursors in biological fluids and tissues of affected patients. BCAA: branched-chain amino acids; BCATc: cytosolic branched-chain aminotransferase; BCATm: mitochondrial branched-chain aminotransferase; BCKA: branched-chain α-keto acids; BCKDH: branched-chain α-keto acid dehydrogenase complex; α-KIC: α-ketoisocaproic acid; α-KIV: α-ketoisovaleric acid; α-KMV: α- α-Keto-β-methylvaleric acid; MSUD: maple syrup urine disease.

(Allahwala et al., 2021; Cheng et al., 2017; Kathait et al., 2018). Vasogenic-interstitial edema in unmyelinated areas of the frontal and temporal lobes were also described (Ha et al., 2004). In addition, cerebral atrophy is observed in patients who experienced prolonged amino acid imbalance during infancy or long periods of poor longitudinal metabolic control along disease progression (Muelly et al., 2013; Schönberger et al., 2004).

Post-mortem studies confirmed the cerebral magnetic resonance imaging observed in the alive patients, revealing extensive brain edema and generalized spongy appearance of the white matter associated with defective myelination, which were more pronounced in subcortical areas, basal ganglia, internal capsule, dental nuclei, cerebellum and brain stem (Kamei et al., 1992; Martin and Schlote, 1972; Menkes et al., 1965). Neuronal abnormalities in the cerebral cortex (Kamei et al., 1992), astrocyte swelling in the corona radiata of the cerebral hemispheres (Menkes et al., 1954), astrogliosis and decrease of oligodendrocyte number in cerebral and cerebellar white matter were also observed (Crome et al., 1961; Menkes et al., 1965; Silberman et al., 1961).

Mounting evidence indicates brain-specific toxic roles for the BCAA and the BCKA. In this particular, crises of metabolic decompensation, which are characterized by high circulating levels of leucine and α-KIC, are usually associated with neurological symptoms worsening, suggesting that cerebral accumulation of these metabolites plays a central role in MSUD neuropathology (Chuang et al., 2019).

In this review, we will update the present knowledge showing that brain amino acid imbalance leads to lower protein and neurotransmitter synthesis and probably contributes to the neuropsychiatric symptoms of MSUD patients (Muelly et al., 2013). We will also focus on the toxic role caused by the BCAA and BCKA though induction of redox homeostasis disruption, disturbance of bioenergetics, and neuroinflammation.

2. Pathomechanisms of neurodegeneration in MSUD patients

Multiple mechanisms seem to be implicated in MSUD neurodegeneration. High blood concentrations of BCAA, especially leucine, associated with low levels of the other large neutral amino acids (LNAA), were shown to cause decrease of cerebral concentrations of essential amino acids, and consequently lower protein and neurotransmitter (dopamine, serotonin, and other amino acid-derived neurotransmitters) synthesis in the CNS (Strauss et al., 2020a). Moreover, cerebral accumulation of α-KIC leads to reduction of the quantities of the excitatory amino acid glutamate (Glu), and its by-products γ-aminobutyric acid (GABA), and glutamine (Gln) (McKenna et al., 1998; Yudkoff et al., 1994), therefore potentially disrupting glutamatergic and GABAergic neurotransmission, as well as the glutamate/glutamine (Glu/Gln) cycle.

Neurotoxicity of the accumulating BCAA and BCKA has also been associated with the neuropathological findings of MSUD patients. This is supported by *in vivo* experimental studies showing neurotoxic effects of α-KIC and of a BCAA mixture. Thus, intrahippocampal injection of α-KIC to developing rats caused neuronal apoptosis (Jouvet et al., 2000a). Furthermore, acute subcutaneous administration of a BCAA mixture activated apoptotic signaling pathways, by increasing the levels of Bax/Bcl-2 ratio and caspase-3 activity in the cerebral cortex, and of caspase-3 and caspase−8 in the hippocampus (Vilela et al., 2017). Morphological alterations and cell death were also shown in primary cultured astrocytes, oligodendrocytes and neuronal cells, as well as in C6 astroglial, neuroblastoma and pheochromocytoma cells (PC12) exposed to these compounds (Contrusciere et al., 2010; de Lima Pelaez et al., 2007; Funchal et al., 2004, 2005, 2006a; Görtz et al., 2003; Jouvet et al., 2000a; Kasinski et al., 2004). Another study revealed that BCAA and BCKA mixtures trigger apoptosis in skin fibroblasts from a MSUD patient (Jouvet et al., 2000b) (Table 1).

2.1. BCAAs and neurotransmitters

Table 2 and Fig. 2 show changes of amino acids and neurotransmitters concentrations in patients and animal models of MSUD. Marked elevations of BCAA and BCKA, particularly leucine and α-KIC, and decreases of phenylalanine, tyrosine, tryptophan, methionine and alanine levels, were found in plasma (Barschak et al., 2007, 2009; Kamei et al., 1992; Morton et al., 2002; Nyhan et al., 1998; Scaini et al., 2018; Wajner et al., 2000) and cerebrospinal fluid (CSF) (Shigematsu et al., 1983; Voyce et al., 1964; Wajner et al., 2000) of untreated MSUD patients, especially during crises of metabolic decompensation. Noteworthy, the reduced plasma levels of these amino acids returned to normal concomitantly with the regularization of the BCAA concentrations when patients were clinically well (Wajner et al., 2000). Moreover, the plasma

Table 1
Morphological and biochemical alterations in brain, neural cells and fibroblasts caused by the branched-chain amino acids and branched-chain α-keto acids accumulated in maple syrup urine disease (MSUD).

	Samples	*In vivo* effects of BCAA and BCKA on apoptotic biomarkers in the brain	References
Acute subcutaneous administration of a BCAA mixture to rats	Cerebral cortex Hippocampus	↑ Bax/Bcl-2 ratio and caspase-3 activity ↑ Caspase-3 and caspase−8 activities	Vilela et al. (2017)
Acute intrahippocampal administration of KIC to rats	Hippocampus	Neuronal apoptosis (DNA fragmentation)	Jouvet et al. (2000a)
	Samples	***In vitro* effects of BCAA and BCKA on neural cell morphology and death**	
Leucine, Isoleucine, valine	Pheochromocytoma cells (PC12)	Cell death (chromatin condensation in the nucleus)	Kasinski et al. (2004)
Leucine Valine	C6 astroglial cells and cortical astrocytes	Cell morphological alterations and death	de Lima Pelaez et al. (2007); Funchal et al. (2005)
	Cortical neuronal cells	Decreased neuronal activity	Görtz et al., 2003
	Mixed cortical astrocytes/ neurons	Apoptosis (condensation of chromatin and nuclei fragmentation)	Contrusciere et al. (2010)
α-KIC	C6 astroglial cells and cortical astrocytes	Cell death (decrease of MTT reduction). Apoptosis and morphological alterations (reduced cytoplasmic volume, nuclear pyknosis and increased caspase activity). Cell morphological alterations and death	Jouvet et al. (2000a) Funchal et al. (2004), 2006a
	Neuroblastoma cells and oligodendrocytes	Cell death (decrease of MTT reduction)	Jouvet et al. (2000a)
	Cortical neuronal cells	Reduction of neuronal activity (electrophysiology)	Görtz et al., 2003
α-KMV and α-KIV	C6 astroglial cells and cortical astrocytes	Cell death (decrease of MTT reduction) Cell morphological alterations and death	Jouvet et al. (2000a) Funchal et al. (2004), 2006a
BCAA and BCKA mixtures	MSUD fibroblasts	Apoptosis (cytoplasmic shrinkage, nuclear pyknosis and decrease of MTT reduction)	Jouvet et al. (2000b)

BCAA: branched-chain amino acids; BCKA: branched-chain α-keto acids; α-KIC: α-ketoisocaproic acid; α-KIV: α-ketoisovaleric acid; α-KMV: α-keto-β-methylvaleric acid; MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium bromide.

Table 2
Amino acid and neurotransmitter imbalance in patients and animal models of maple syrup urine disease (MSUD).

MSUD patients			
	Samples	Amino acid and neurotransmitter imbalance	References
	Plasma	↑ BCAA levels ↑ BCKA levels ↑ Alloisoleucine levels ↓ Tryptophan and methionine levels ↓ Phenylalanine, tyrosine, tryptophan and methionine levels ↓ Alanine levels	Barschak et al. (2007); Morton et al. (2002); Wajner et al. (2000) Barschak et al. (2009) Barschak et al. (2007), 2009; Kamei et al. (1992); Scaini et al. (2018) Barschak et al. (2009) Wajner et al. (2000) Morton et al. (2002); Nyhan et al. (1998)
	CSF	↑ BCAA levels ↑ BCKA levels ↓ Phenylalanine, tyrosine, tryptophan and methionine levels	Shigematsu et al. (1983); Voyce et al. (1964); Wajner et al. (2000) Shigematsu et al. (1983) Wajner et al. (2000)
	Brain	↑ BCAA and BCKA concentrations ↑ BCAA concentrations; ↓ Glutamate, GABA and glutamine concentrations ↓ Glutamate concentrations	Jan et al., 2003; Terek et al. (2013) Prensky and Moser (1966) Muelly et al. (2013)
Genetic animal models of MSUD			
Genetic intermediate mouse model	Brain	↑ BCAA concentrations ↑ α-KIC concentrations ↓ Serine, alanine, glutamate, glutamine, aspartate, GABA, dopamine and serotonin concentrations ↓ Tyrosine, tryptophan, glutamate, aspartate, GABA and dopamine concentrations	Skvorak et al. (2009); Zinnanti et al. (2009) Zinnanti et al. (2009) Skvorak et al. (2009) Zinnanti et al. (2009)
Genetic Zebrafish model	Brain	↓ Glutamate, glutamine and GABA concentrations	Friedrich et al. (2012)
Genetic *Drosophila* model	Brain	↓ Glutamate concentrations	Tsai et al. (2020)
MSUD neonatal calves		↑ BCAA concentrations; ↓ Glutamate, aspartate and GABA concentrations	Dodd et al. (1992)
Chemical rat models of MSUD: *In vivo* effects of leucine			
Acute subcutaneous administration of leucine to rats	Plasma	↓ Serine, histidine, alanine, tyrosine, methionine, phenylalanine, isoleucine and valine levels	Araújo et al. (2001)
	Brain	↓ Methionine, phenylalanine, isoleucine and valine concentrations	
Chemical rat models of MSUD: *In vitro* effects of α-KIC			
α-KIC			

Table 2 (*continued*)

MSUD patients			
	Samples	Amino acid and neurotransmitter imbalance	References
	Cultured astrocytes	↓ Glutamine synthesis by glutamine synthetase (labeled [^{15}N]) due to reduced glutamate concentrations ↓ Aspartate synthesis from glutamate (labeled [^{13}C])	Yudkoff et al. (1994) McKenna et al. (1998)

BCAA: branched-chain amino acids; CSF: cerebrospinal fluid; GABA: Gamma-aminobutyric acid; α-KIC: α-ketoisocaproic acid; α-KIV: α-ketoisovaleric acid; α-KMV: α-keto-β-methylvaleric acid; MSUD: maple syrup urine disease.

amino acid profile observed in untreated patients was similar to that of nonadherent treated MSUD patients (Barschak et al., 2009). The reduced circulating concentrations of the LNAA were attributed at least in part to their sequestration and possible accelerated catabolism in the peripheral tissues due to a competition with the high intracellular concentrations of the BCAA for their efflux to the blood (de Cespedes et al., 1989). Trans-stimulation of L-system amino acid transport, which regulates the intracellular LNAA concentrations by exchanging intracellular for extracellular amino acids that share this membrane carrier system, may also explain the low circulating levels of LNAA (Christensen, 1990). In this context, high tissue concentrations of leucine and the other BCAA could be exchanged by the LNAA, decreasing their concentrations in the circulation. These possibilities are supported by a previous study demonstrating that intravenous injection of leucine alone or of a BCAA mixture to healthy individuals resulted in a significant decrease of plasma levels of tyrosine, phenylalanine and methionine (Eriksson et al., 1981). Similar data were obtained after a single subcutaneous injection of leucine to developing rats that resulted in significant reductions of the blood levels of phenylalanine, tyrosine, isoleucine, valine, methionine, alanine, serine and histidine (Araújo et al., 2001). The same study showed decreased brain concentrations of methionine, phenylalanine, isoleucine and valine, and besides that leucine markedly inhibits phenylalanine and lysine incorporation into brain proteins. It is emphasized that LNAA carrier system (LAT1/SLC7A5) at the blood-brain barrier (BBB) is almost totally saturated at normal blood amino acid concentrations, and about 50% saturated with leucine and phenylalanine alone under physiological conditions (Smith et al., 1987). Thus, the highly increased plasma leucine concentrations found in MSUD would saturate this transporter, impairing the influx of other essential amino acids that share the same carrier into the brain, compromising cerebral protein and neurotransmitter synthesis. The significant reduction of LNAA levels in CSF of MSUD patients during crises (Wajner et al., 2000) and the lower concentrations of the neurotransmitters serotonin, dopamine, norepinephrine and histamine (Fig. 2) (Table 2) (Araújo et al., 2001; Boado et al., 1999; Killian and Chikhale, 2001; Strauss et al., 2020a) corroborate with this hypothesis.

Reduction of the amounts of the neurotransmitters Glu and GABA, as well as Gln, has been also observed in brain of MSUD patients (Muelly et al., 2013; Prensky and Moser, 1966; Yudkoff et al., 2005; Zinnanti et al., 2009), and more important diminution of cerebral concentrations of Glu and N-acetylaspartate were related to the neuropsychiatric symptoms observed in these individuals (Muelly et al., 2013). Decreased Glu, GABA, and aspartate concentrations were also observed in brains of calves with naturally occurring BCKDH deficiency (Dodd et al., 1992). In regards to the murine model of MSUD, α-KIC accumulation and depletion of tyrosine, tryptophan, aspartate, Glu, GABA, pyruvate, threonine, alanine, serotonin and dopamine, and increase of α-ketoglutarate and lactate were detected in brain of these animals (Skvorak et al., 2009; Zinnanti et al., 2009). Interestingly, these changes were associated with

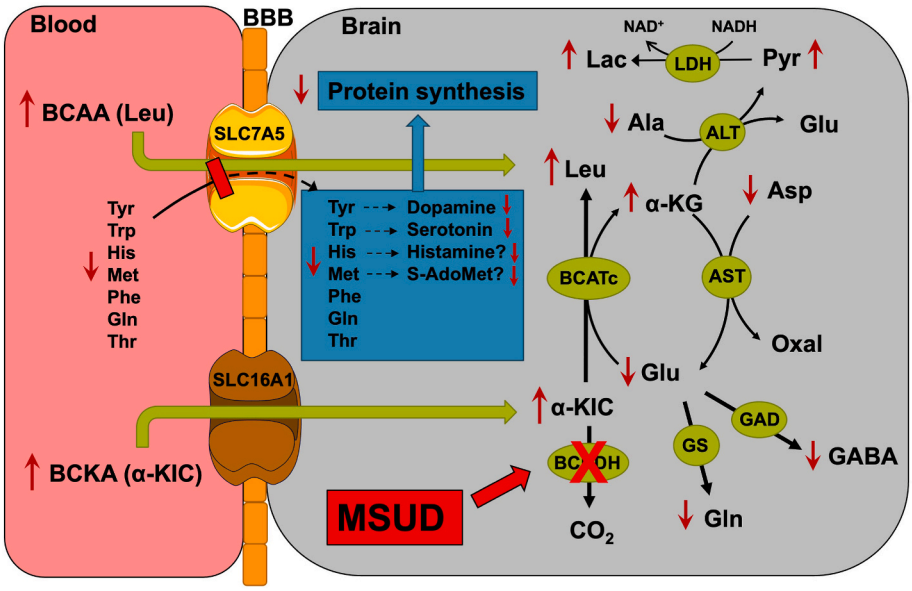

Fig. 2. Amino acid and neurotransmitter imbalance in MSUD. Deficiency of BCKDH activity results in elevated blood BCAA levels that impairs cerebral uptake of large neutral amino acids through the BBB that use the same transporter (SLC7A5), decreasing their concentrations in the brain, as well as protein synthesis and formation of their by-products, such as neurotransmitters and S-AdoMet. Decreased efflux of large neutral amino acids in peripheral tissues due to a competition for the amino acid L transporter results in lower levels of these amino acids in blood, further aggravating their brain deficit. Intracerebral α-KIC accumulation causes decrease of Glut and increase of α-KG levels by reverse transamination (BCATc). Decreased production of Gln and GABA from Glu follows, as well as of Ala and Asp depletion by α-KG transamination, simultaneously with Pyr and subsequently Lac synthesis. Ala: alanine; Asp: aspartate; BBB: blood-brain barrier; BCATc: cytosolic branched-chain aminotransferase; BCKDH: branched-chain α-keto acid dehydrogenase complex; GABA: γ-aminobutyric acid; GAD: glutamate decarboxylase; Glu: glutamate; Gln: glutamine; GS: glutamine syntethase; His: histidine; α-KG: α-ketoglutarate; α-KIC: α-ketoisocaproic acid; Lac: lactate; Leu: leucine; Met: methionine; MSUD: maple syrup urine disease; Oxal: oxaloacetate; Phe: phenylalanine; Pyr: pyruvate; S-AdoMet: S-adenosyl-methionine; Thr: threonine; Trp: tryptophan; Tyr: tyrosine.

reduction of brain weight, striatal changes, dystonia and gait abnormalities (Zinnanti et al., 2009). Similar findings were demonstrated in the brain and spinal cord of the zebrafish MSUD model (Friedrich et al., 2012) and in the genetic *Drosophila* model of MSUD (Tsai et al., 2020).

Most of these alterations have been attributed to augmented brain concentrations of α-KIC generated in MSUD by mitochondrial transamination of leucine in astrocytes (rodent) or capillary endothelial cells (human), and also by α-KIC entrance from the circulation into the brain by the BBB monocarboxylate transporter (MCT/SLC16A1) (Boado et al., 1999; Killian and Chikhale, 2001; Sperringer et al., 2017; Yudkoff et al., 2005). As can be seen in the figure, α-KIC leads to a depletion of Glu, GABA, Gln, aspartate and alanine, as well as an overproduction of α-ketoglutarate and lactate in the brain (Fig. 2) (McKenna et al., 1998; Yudkoff et al., 1994). Aspartate depletion leads to a decrease of cerebral N-acetylaspartate concentrations in brain of MSUD patients (Muelly et al., 2013), and to a failure of the malate–aspartate shuttle, which transfers glycolytically-derived reducing equivalents from cytosol to mitochondria (Llorente-Folch et al., 2013). Excessive cytosolic reducing equivalents, in conjunction with inhibition of mitochondrial electron flow through the respiratory chain caused by the BCKA and BCAA (Ribeiro et al., 2008; Sgaravatti et al., 2003), result in a rise of brain lactate levels from pyruvate (Felber et al., 1993; Jan et al., 2003; Srinivasan et al., 2009; Terek et al., 2013; Yudkoff et al., 2005). Disruption of the malate-aspartate shuttle may also impair oxidative phosphorylation (McKenna et al., 2006), reducing ATP synthesis in neurons (Llorente-Folch et al., 2013; Yudkoff et al., 2005).

BCKA and BCAA were also shown to markedly reduce synaptic Na^+, K^+-ATPase activity in rat brain *in vitro* (Wajner et al., 2007) and *in vivo* (Rosa et al., 2016). Of note, Na^+, K^+-ATPase accounts for approximately 50% of brain ATP consumption, highlighting its importance for normal cerebral functioning (Erecinska and Silver, 1994; Erecinska et al., 2004). Moderate inhibitions of Na^+, K^+-ATPase activity were shown to deregulate intra and extracellular concentrations of Na^+ and K^+ (Bélanger et al., 2011; Erecinska et al., 2004), which may be presumably involved in the cerebral edema observed in MSUD patients. Brain ion imbalance also leads to disruption of membrane potential and Na^+-dependent transport of glucose, amino acids and neurotransmitters (Geering, 1990). Na^+, K^+-ATPase has also an important role in Glu uptake from the synaptic cleft by astrocytes (Andersen et al., 2021; Passlick et al., 2021), and reducing the activity of this ion pump is known to cause seizures (Freitas et al., 2018; McDonald et al., 2018). Therefore, BCKA/BCAA-induced inhibition of Na^+, K^+-ATPase activity possibly contribute to the seizures observed in MSUD patients, especially during episodes of metabolic decompensation characterized by high concentrations of these metabolites.

On the other hand, brain reduction of Glu concentrations alters synaptic plasticity, potentially disturbing learning and memory and causing psychiatric symptoms in MSUD patients (Barnes et al., 2020; McEntee and Crook, 1993; Muelly et al., 2013; Strauss et al., 2020a). Furthermore, low brain levels of N-acetylaspartate were associated with anxiety, depression and attention-deficit hyperactivity disorder (Muelly et al., 2013). Disruption of malate-aspartate shuttle was also associated with infantile-onset epileptic encephalopathies (van Karnebeek et al., 2019) and autism (Aoki and Cortese, 2016).

Methionine levels, which are also decreased in plasma and brain of patients (Barschak et al., 2009; Wajner et al., 2000) and in animal models of MSUD (Araújo et al., 2001), may lead to a reduction of S-adenosylmethionine (S-AdoMet), a major methyl group donor in the human brain (Dash et al., 2016; Saunderson et al., 2016). Low amounts of S-AdoMet may therefore deregulate the structure and function of a large number of brain cell components, such as DNA, RNA, lipids, proteins, amino acids, neurotransmitters and guanidinoacetate (Loenen, 2006). In addition, decreased brain methionine concentrations possibly compromise the antioxidant defences in MSUD since methionine is needed for the synthesis of the antioxidants cysteine and reduced glutathione (GSH) (Medina et al., 2022), increasing therefore the susceptibility of patients to free radical attack and oxidative stress.

2.2. *BCAA and BCKA neurotoxicity*

2.2.1. *Bioenergetics disruption*

Growing evidence indicates bioenergetics dysregulation in MSUD. In this scenario, increased concentrations of lactate (Felber et al., 1993; Jan et al., 2003; Srinivasan et al., 2009; Terek et al., 2013) and diminished creatine levels were found in brain of MSUD patients. Lactic acidosis/aciduria was also observed during metabolic crises in these patients (Srinivasan et al., 2009; Muelly et al., 2013; Yang et al., 2019) (Table 3). The decreased NAD^+/NADH ratio, citrate synthase and pyruvate

Table 3
Bioenergetics disruption in patients and animal models of maple syrup urine disease (MSUD): role of the accumulating branched-chain amino acids and branched-chain α-keto acids.

MSUD patients			
	Samples	Bioenergetics disruption	References
	Blood/urine	Lactic acidosis/ lactic aciduria	Yang et al. (2019)
	Brain	↑ Lactate concentrations	
		↓ N-acetylaspartate and creatine concentrations	Felber et al. (1993); Jan et al. (2003); Srinivasan et al. (2009); Terek et al. (2013) Muelly et al. (2013); Srinivasan et al. (2009)
	Fibroblasts	↓ NAD^+/NADH ratio, citrate synthase and pyruvate dehydrogenase-E2 activities, ATP concentrations, sirtuin 4 and mitochondrial biogenesis proteins	Strand et al. (2014)
Genetic intermediate mouse model of MSUD			
	Brain	↓ ATP, phosphocreatine and pyruvate concentrations ↑ Lactate and α-ketoglutarate concentrations	Zinnanti et al. (2009)
Chemical rat models of MSUD: *In vivo* effects of BCAA and BCKA			
Acute or chronic subcutaneous administration of leucine to rats	Midbrain and cerebellum	↓ CK activity	Pilla et al. (2003b)
Intracerebroventricular injection of α-KIC to rats	Hippocampus	↓ Complexes I and II-III activities	Farias et al. (2021)
Chemical rat models of MSUD: *In vitro* effects of BCAA and BCKA			
Leucine	Cerebral cortex	↓ CO_2 production from glucose, acetate and citrate (labeled [^{14}C]) and complex IV activity; ↑ Glucose uptake	Ribeiro et al. (2008)
		↓ CK activity	Pilla et al. (2003a)
	Midbrain and cerebellum	↓ CK activity	Pilla et al. (2003a)
Isoleucine and Valine	Cerebral cortex	↓ CO_2 production from acetate (labeled [^{14}C]) and complexes II-III, III and IV activities; ↑ Glucose uptake	Ribeiro et al. (2008)
		↓ CK activity	Pilla et al. (2003a)
	Midbrain and cerebellum	↓ CK activity	Pilla et al. (2003a)
α-KIC	Forebrain	↓ α-KGDH and PDH activities	Patel et al. (1973); Patel (1974)
		↓ Mitochondrial pyruvate transport (labeled [^{14}C])	
		↑ Oxygen consumption in state 4 (uncoupling behavior)	
		↓ NAD(P)H levels, mitochondrial membrane potential, state 3 respiration and ADP/O ratio (metabolic inhibition)	Halestrap et al. (1974) Amaral et al. (2010)
	Cerebral cortex	↓ CO_2 production from acetate (labeled [^{14}C]) and complex I-III activity; ↑ Lactate release and glucose uptake	Sgaravatti et al. (2003)
		↓ PDH activity	Ribeiro et al. (2008)
	C6 astroglial cells and primary cortical astrocytes	↓ CK activity ↑ Lactate synthesis from glutamate (labeled [^{13}C])	Funchal et al. (2004), 2006b McKenna et al. (1998)
	Hippocampal neuronal cells	↓ MTT reduction reflecting mitochondrial dehydrogenases inhibition	Farias et al. (2021)
α-KMV	Brain	↓ α-KGDH and PDH activities	Patel et al. (1973); Patel (1974)
	Cerebral cortex	↓ CO_2 production from acetate (labeled [^{14}C]) and complex I-III activity; ↑ Lactate release and glucose uptake	Sgaravatti et al. (2003)
	C6 astroglial cells	↓ CK activity	Funchal et al. (2006b)
α-KIV	Brain	↓ α-KGDH and PDH activities	Patel et al. (1973); Patel (1974)
	Cerebral cortex	↓ CO_2 production from acetate (labeled [^{14}C]) and complex I-III activity; ↑ Lactate release and glucose uptake	Sgaravatti et al. (2003)
	C6 astroglial cells and primary cortical astrocytes	↓ CK activity	Funchal et al. (2004), 2006b

BCAA: branched-chain amino acids; BCKA: branched-chain α-keto acids; CK: creatine kinase; α-KGDH: α-ketoglutarate dehydrogenase; α-KIC: α-ketoisocaproic acid; α-KIV: α-ketoisovaleric acid; α-KMV: α-keto-β-methylvaleric acid; MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium bromide; PDH: pyruvate dehydrogenase; PDH-E2: pyruvate dehydrogenase-E2; SIRT4: sirtuin 4.

dehydrogenase (PDH)-E2 activities, as well as ATP levels, sirtuin 4 and altered mitochondrial biogenesis found in fibroblasts of affected patients further support disruption of mitochondrial bioenergetics in MSUD (Strand et al., 2014). Elevated lactate and α-ketoglutarate concentrations, as well as decreased levels of pyruvate, phosphocreatine, ATP were also found in the brain of a mouse model of MSUD (Zinnanti et al.,

2009), corroborating the bioenergetics dysregulation findings observed in humans affected by the disease.

In regards to the underlying causes of bioenergetics failure in MSUD, it has been shown that the accumulated BCAA and BCKA compromise the citric acid cycle activity, increase anaerobic glycolysis and inhibit various respiratory chain complexes activities in brain of infant and adolescent rats (Chuang et al., 2019; Ribeiro et al., 2008; Sgaravatti et al., 2003). α-KIC was also shown to decrease the activities of α-keto-glutarate dehydrogenase (α-KGDH) and PDH in rat and human brain (Patel et al., 1973; Patel, 1974), as well as the transport of pyruvate into rat brain mitochondria (Halestrap et al., 1974). Additional studies revealed that α-KIC markedly disturbs Glu plus malate-supported mitochondrial respiration, by increasing state 4 respiration (uncoupled behavior) and decreasing state 3 respiration (metabolic inhibitor), ADP/O ratio, NAD(P)H levels and mitochondrial membrane potential in rat brain (Amaral et al., 2010). α-KIC-induced inhibition of 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) reduction, reflecting decreased mitochondrial dehydrogenase activities, was also found in hippocampal neuronal cells (Farias et al., 2021). Cellular ATP buffering might be potentially disturbed in the brain by BCAA and BCKA, since these compounds were shown to significantly reduce creatine kinase activity in rat cerebral cortex, midbrain and cerebellum (Pilla et al., 2003a), as well as in cultured cortical astrocytes (Funchal et al., 2004) and C6 astroglial cells (Funchal et al., 2006b). *In vivo* acute and chronic subcutaneous administration of leucine was also shown to reduce creatine kinase activity in midbrain and cerebellum of rats (Pilla et al., 2003b), whereas an intracerebroventricular injection of α-KIC decreased the activities of complexes I and II-III of the respiratory chain in rat hippocampus (Farias et al., 2021), therefore supporting the *in vitro* studies.

Altogether, the above data obtained from patients and from the genetic murine model of MSUD, in conjunction with the *in vitro* and *in vivo* experimental studies carried out in rat models, strongly indicate that brain disruption of mitochondrial bioenergetics elicited by BCAA and BCKA ensues in MSUD. Since the brain is a mitochondria-enriched tissue extremely dependent on oxidative metabolism to support its high energy demand (Mergenthaler et al., 2013; Rolfe and Brown, 1997), it is suggested that bioenergetics impairment may represent an important pathomechanism contributing to the neurological symptoms and cerebral damage in this intoxicating metabolic disorder.

2.2.2. Oxidative stress

Oxidative stress is a deleterious process usually due to excessive reactive species production that cannot be overcome by the cellular antioxidant system (Halliwell and Gutteridge, 2015). This condition causes oxidative damage of critical biomolecules, impairing cell functioning and potentially inducing cell death (Angelova and Abramov, 2018; Figueira et al., 2013). Brain is highly vulnerable to oxidative stress because of its high rate of mitochondrial oxidative metabolism leading to increased ROS production, as well as due to its high iron content that facilitates the Fenton reaction, a major source of the very toxic hydroxyl radical, and the large content of polyunsaturated lipids highly vulnerable to oxidation (Al-Gubory and Garrel, 2016; Mori et al., 2007). The reduced antioxidant capacity of the brain, reflected by decreased GSH levels and low activities of the antioxidant enzymes glutathione peroxidase (GPX), glutathione reductase (GR), catalase (CAT) and superoxide dismutase (SOD), further makes this tissue more susceptible to redox homeostasis alterations (Al-Gubory and Garrel, 2016; Mori et al., 2007).

Table 4 exhibits biochemical data on redox status in biological fluids of patients and in brain of animal models of MSUD, supporting a role for oxidative stress in the pathophysiology of this disease. Thus, increased malondialdehyde (MDA) levels (lipid oxidation) and protein carbonyl content (protein oxidative damage), as well as reduced antioxidant reactivity (TAR) and selenium levels (compromised antioxidant defences), were found in plasma of MSUD patients (Barschak et al., 2006, 2007, 2008a, 2008b; Mescka et al., 2013). Parameters of oxidative stress

Table 4
Disruption of redox homeostasis in patients and in genetic and chemical models of maple syrup urine disease (MSUD): role of the accumulating branched-chain amino acids and branched-chain α-keto acids.

MSUD patients	Samples	Oxidative stress parameters	References
	Plasma	↑ MDA levels (lipid oxidative damage)	Barschak et al. (2006), 2008a, 2008b; Mescka et al. (2013)
		↑ Protein carbonyl (protein oxidative damage) and ↓ L-carnitine levels	Mescka et al. (2013)
		↓ TAR (impaired antioxidant system)	Barschak et al. (2006), 2008a, 2008b
		↓ Selenium levels	Barschak et al. (2007)
	Peripheral leukocytes	↑ DNA oxidative damage by the comet assay	Mescka et al. (2015a)
	Erythrocytes	↓ GPx activity (impaired enzymatic antioxidant defenses)	Barschak et al. (2007)
	Urine	↑ F-2 isoprostanes (lipid oxidative damage) and di-tyrosine (protein oxidative damage) levels	Guerreiro et al. (2015); Mc Guire et al. (2009)
		↑ 8-OHdG (DNA oxidative damage)	Hauschild et al. (2019)
		↓ Antioxidant capacity (impaired antioxidant defenses)	Guerreiro et al. (2015)
Genetic *Drosophila* model of MSUD			
	Brain	↑ MDA levels (lipid oxidative damage)	Tsai et al. (2020)
Chemical rat models of MSUD: *In vivo* effects of the BCAA and BCKA			
Acute subcutaneous administration of a BCAA mixture to rats	Cerebral cortex	↑ TBA-RS levels (lipid oxidative damage), carbonyl content and sulfhydryl oxidation (protein oxidative damage) ↓ GPx and CAT activities (impaired enzymatic antioxidant defenses)	Mescka et al. (2011)
	Hippocampus	↑ DNA oxidative damage by the comet assay	Scaini et al. (2012)
Chronic subcutaneous administration of a BCAA mixture to rats	Cerebral cortex	↑ TBA-RS levels (lipid oxidative damage), DCFH oxidation (ROS and RNS production) and CAT activity ↓ GSH levels, SOD, GPx and G6PD activities (impaired antioxidant defenses)	Mescka et al. (2016)
	Cerebellum	↑ TBA-RS levels (lipid oxidative damage)	Mescka et al. (2016)
		↑ Carbonyl content (protein oxidative damage)	

(*continued on next page*)

Table 4 (*continued*)

MSUD patients	Samples	Oxidative stress parameters	References
	Striatum	↑ TBA-RS levels (lipid oxidative damage), DCFH oxidation (ROS and RNS production) and SOD activity	Wessler et al. (2020)
			Scaini et al. (2012)
		↑ DNA oxidative damage by the comet assay	
	Hippocampus	↑ TBA-RS levels (lipid oxidative damage) and sulfhydryl oxidation (protein oxidative damage); ↓ CAT	Wessler et al. (2020) Scaini et al. (2012)
		↑ DNA oxidative damage by the comet assay	
Intracerebroventricular injection of α-KIC to rats	Cerebral cortex	↑ MDA levels (lipid oxidative damage), carbonyl content (protein oxidative damage) and DNA oxidative damage by the comet assay	Taschetto et al. (2017)
	Striatum	↑ MDA levels (lipid oxidative damage), carbonyl content (protein oxidative damage), DNA oxidative damage by the comet assay and SOD activity	
		↓ CAT activity	Taschetto et al. (2017)
	Hippocampus	↑ MDA levels (lipid oxidative damage), carbonyl content (protein oxidative damage), DNA oxidative damage by the comet assay and SOD activity	Taschetto et al. (2017)
		↓ CAT activity (impaired enzymatic antioxidant defenses)	Farias et al. (2021)
		↑ DCFH oxidation (ROS and RNS production)	
Chemical rat models of MSUD: *In vitro* effects of BCAA and BCKA			
Leucine	Cerebral cortex	↑ Chemiluminescence and TBA-RS levels (lipid oxidative damage)	Bridi et al. (2003)
		↓ TAR and TRAP (impaired antioxidant defenses)	
	C6 astroglial cells	↑ Nitric oxide levels (RNS production) and ↓ GSH levels (impaired antioxidant system)	de Lima Pelaez et al. (2007)
	Human leukocytes	↑ DNA oxidative damage by the comet assay	Hauschild et al. (2019);
Isoleucine	Cerebral cortex	↑ Chemiluminescence (lipid oxidative damage)	Mescka et al. (2014)
		↓ TRAP (impaired antioxidant defense)	Bridi et al. (2003)
	C6 astroglial cells	↑ Nitric oxide levels (RNS production)	
		↓ GSH levels (impaired antioxidant defense)	de Lima Pelaez et al. (2007)
	Human leukocytes	↑ DNA oxidative damage by the comet assay	Hauschild et al. (2019)
Valine	Cerebral cortex	↓ TRAP (impaired antioxidant defense)	Bridi et al. (2003)
	C6 astroglial cells	↑ Nitric oxide levels (RNS production)	
		↓ GSH levels (impaired antioxidant defense)	de Lima Pelaez et al. (2007)
	Human leukocytes	↑ DNA oxidative damage by the comet assay	Hauschild et al. (2019)
α-KIC	Cerebral cortex	↑ Chemiluminescence and TBA-RS levels (lipid oxidative damage)	Bridi et al. (2005)
		↓ TAR, TRAP and GPx activity (impaired antioxidant defenses)	
	C6 astroglial cells	↑ Nitrites levels (RNS production)	
		↓ TAR, GSH levels, GPx and SOD activities (impaired antioxidant defenses)	Funchal et al. (2006a)
	Human leukocytes	↑ DNA oxidative damage by the comet assay	Hauschild et al. (2019); Mescka et al. (2014)
	Hippocampal neuronal cells	↑ DCFH oxidation (ROS and RNS production)	Farias et al. (2021)
α-KMV	Cerebral cortex	↑ Chemiluminescence (lipid oxidative damage)	Bridi et al. (2005)
	C6 astroglial cells	↑ Nitrites levels (RNS production)	Funchal et al. (2006a)
		↓ TAR, GSH levels and SOD activity (impaired antioxidant defenses)	
	Human leukocytes	↑ DNA oxidative damage by the comet assay	Hauschild et al. (2019)
α-KIV	Cerebral cortex	↑ Chemiluminescence (lipid oxidative damage)	
		↓ TAR and TRAP (impaired antioxidant defenses)	Bridi et al. (2005)
	C6 astroglial cells	↑ Nitrites levels (RNS production)	Funchal et al. (2006a)
		↓ TAR and GSH levels (impaired antioxidant defenses)	
	Human leukocytes	↑ DNA oxidative damage by the comet assay	Hauschild et al. (2019)
Alloisoleucine	Human leukocytes	↑ DNA oxidative damage by the comet assay	Hauschild et al. (2019)
BCAA mixture	Primary cortical microglia	↑ F-2 isoprostanes levels (lipid oxidative damage)	De Simone et al. (2013)

BCAA: branched-chain amino acids; BCKA: branched-chain α-keto acids; CAT: catalase; DCFH: 2′,7′-Dichlorofluorescein; G6PD: glucose 6-phosphate

dehydrogenase; GPx: glutathione peroxidase; GSH: reduced glutathione; 8-OHdG: 8-Hydroxy-2′-deoxyguanosine; α-KIC: α-ketoisocaproic acid; α-KIV: α-ketoisovaleric acid; α-KMV: α-keto-β-methylvaleric acid; MDA: malondialdehyde; SOD: superoxide dismutase; TAR: total antioxidant reactivity; TBA-RS: thiobarbituric acid-reactive substances; TRAP: total radical-trapping antioxidant capacity.

in the urine, including high amounts of F-2 isoprostanes and di-tyrosine (by-products of lipid and protein oxidation, respectively) (Guerreiro et al., 2015; Mc Guire et al., 2009), and low antioxidant capacity (Guerreiro et al., 2015), as well as decreased erythrocyte GPx activity (Barschak et al., 2007) were also observed in MU patients.

L-carnitine concentrations were also found decreased and negatively correlated with MDA values (Mescka et al., 2013) and with di-tyrosine levels in MSUD patients (Guerreiro et al., 2015). Furthermore, L-carnitine supplementation to patients caused a significant drop of MDA plasma levels (Mescka et al., 2013), as well as of the urinary excretion of di-tyrosine and isoprostanes (Guerreiro et al., 2015). Since indirect antioxidant effects have been attributed to L-carnitine (Derin et al., 2004; Gülçin, 2006), it is conceivable that deficit of this endogenous compound may have contributed to the oxidative damage observed in MSUD patients. Further evidence supporting this hypothesis is the observations of a significant reduction of DNA oxidative damage in peripheral blood leukocytes and urine of MSUD patients supplemented with L-carnitine (Hauschild et al., 2019; Mescka et al., 2015a).

Additional studies revealed a positive correlation between leucine levels and the pro-oxidant DNA marker 8-Hydroxy-2′-deoxyguanosine (8-OHdG) in urine of MSUD patients, suggesting that high concentrations of leucine may be implicated in the DNA damage (Hauschild et al., 2019). DNA oxidative damage was also observed in human peripheral leukocytes exposed to leucine, isoleucine, valine, and alloisoleucine, as well as to α-KIC, α-KIV and α-KMV. Noteworthy, L-carnitine partly prevented DNA damage (Hauschild et al., 2019; Mescka et al., 2014). Taken together, these findings support a role of the major metabolites accumulating in MSUD inducing oxidative stress, and reinforce the indirect antioxidant property of L-carnitine. It is emphasized that the effect of add-on L-carnitine on the neurological outcomes of MSUD patients has not yet been studied. However, based on the many beneficial effects of L-carnitine in animal models and its lack of toxicity, clinical studies are now warranted to test its effects in patients.

Neuronal apoptosis associated with lipid peroxidation have been also observed in brain of the *Drosophila* genetic model of MSUD (Tsai et al., 2020), indicating that oxidative stress contributes to neurodegeneration. In this scenario, the BCAA and BCKA accumulated in MSUD induce pronounced oxidative stress *in vivo* and *in vitro* in rat brain (Table 4) (Bridi et al., 2003, 2005; De Simone et al., 2013; Funchal et al., 2006a; de Lima Pelaez et al., 2007; Mescka et al., 2011, 2016; Scaini et al., 2012; Taschetto et al., 2017; Wessler et al., 2020), indicating that disruption of redox status caused by these compounds should be considered a relevant pathomechanism of brain damage in this disorder.

2.2.3. *Pro-inflammatory state*

An increased inflammatory response was also observed in MSUD patients and in animal models of this disorder (Table 5). This is not surprising since oxidative stress and inflammation are interrelated processes (Popa-Wagner et al., 2013). Thus, increased levels of the pro-inflammatory biomarkers interleukin-1β (IL-1β), interleukin-6 (IL-6) and interferon-γ (INF-γ) were found in plasma from MSUD patients (Mescka et al., 2015b). Noteworthy, the concentrations of these pro-inflammatory biomarkers were normalized by L-carnitine supplementation possibly due to the indirect anti-inflammatory properties of this compound (Pertosa et al., 2005; Szefel et al., 2012). Another study showed plasma elevations of the same interleukins, as well as of tumour necrosis factor α (TNF-α), soluble intercellular adhesion molecule-1 (sICAM-1) and soluble vascular cell adhesion molecule-1 (sVCAM-1) (Scaini et al., 2018), further supporting an exacerbated pro-inflammatory response in this disorder. Interestingly, a strong correlation between IL-1β and sICAM-1 levels with the frequency of metabolic crises was also revealed (Scaini et al., 2018), suggesting the contribution of inflammation in the recurrent episodes of metabolic decompensation in MSUD.

Table 5
Increased pro-inflammatory state in patients with maple syrup urine disease (MSUD): role of the accumulating branched-chain amino acids.

MSUD patients			
	Samples	Inflammatory biomarkers	References
	Plasma	↑ IL-1β, IL-6 and INF-γ levels	Mescka et al. (2015b)
		↑ INF-γ, TNF-α, IL-1β, IL-6, sICAM-1 and sVCAM-1 levels	Scaini et al. (2018)
		↑ Cathepsin D levels	Scaini et al. (2017)
Chemical models of MSUD: *In vivo* effects of BCAA			
Acute subcutaneous administration of a BCAA mixture to rats	Cerebral cortex Hippocampus	↑ TNF-α, IL-6 and IL-1β concentrations ↑ IL-6, INF-γ and TNF-α; ↓ IL-10 concentrations	Rosa et al., (2016); Wessler et al., (2019)

BCAA: branched-chain amino acids; INF-γ: interferon-γ; IL-1β: interleukin 1β; IL-6: interleukin 6; M IL-10: interleukin 10; sICAM-1: soluble intercellular adhesion molecule-1; sVCAM-1: soluble vascular cell adhesion molecule-1; TNF-α: tumor necrosis factor-α.

Increased plasma concentrations of cathepsin D, a lysosomal aspartic protease that regulates the progression of inflammatory processes by inducing inflammatory cytokine secretion, and apoptosis by activating caspases 3 and 9 (Heinrich et al., 2004; Minarowska et al., 2007), were also found in MSUD patients (Scaini et al., 2017). Since cathepsin-D also mediates microglial neurotoxicity, it is suggested that increased levels of this protein indicate microglial activation and neuroinflammation (Kim et al., 2007). Of note, cathepsin-D elevation may be induced by oxidative stress (Kågedal et al., 2001) and by the cytokines INF-γ and TNF-α (Erdmann et al., 2008). No less important is that cathepsin-D upregulation precedes neuronal injury in experimental models of neurodegeneration, indicating its involvement with brain damage (Hetman et al., 1997; Moechars et al., 1999; Wirths et al., 2010; Yelamanchili et al., 2011).

Other studies showed that *in vivo* acute subcutaneous administration of a BCAA mixture to rats caused both BBB breakdown and increased levels of the cytokines TNF-α, IL-6 and IL-1β, as well as decreased concentrations of IL-10 in the hippocampus and cerebral cortex of the animals (Rosa et al., 2016) (Table 5). It was also shown that combined injections of lipopolysaccharide, that induces an inflammatory response, and a BCAA mixture resulted in highly increased levels of INF-γ, TNF-α, IL-6 and IL-1β, and reduced concentration of IL-10 in these cerebral structures (Wessler et al., 2019). It is therefore presumed that the amino acids that most accumulate in MSUD induce a neuroinflammatory response *in vivo*.

It is difficult to project the pathophysiological relevance of high blood levels of pro-inflammatory biomarkers onto the CNS damage in MSUD. However, it is emphasized that concomitant BBB breakdown and increased levels of various pro-inflammatory interleukins were achieved in brain of developing rats following a BCAA mixture injection, that reproduces the blood levels of these amino acids in MSUD (Rosa et al., 2016; Wessler et al., 2019). Furthermore, astrogliosis, which is closely linked to neuroinflammation, represents a common neuropathological finding in the genetic mouse model of MSUD (Zinnanti et al., 2009) and in postmortem brain analysis of MSUD affected patients (Crome et al., 1961; Menkes et al., 1965; Silberman et al., 1961). Therefore, it is conceivable that the increased peripheral inflammatory response identified in MSUD patients may also occur in the CNS, exerting a deleterious role in the cerebral tissues of these patients.

3. Concluding remarks and future directions

Multiple pathomechanisms are presumably involved in MSUD neurodegeneration (Fig. 3). Among them are plasma and brain amino acid imbalance, leading to decreased synthesis of important neurotransmitters and cerebral proteins, and the neurotoxicity of the BCAA and BCKA, especially leucine and α-KIC, which most accumulate in this intoxicating disorder. Recent data indicate that the neurological symptoms and brain abnormalities of MSUD patients may be at least in part due to deleterious roles of BCAA and BCKAs to the CNS. We focused here on the derangements provoked by accumulation of these compounds in the brain of MSUD patients and animal models, exploring the potential mechanisms driving neurologic dysfunction. Insights from animal studies have shown that these metabolites markedly disrupt redox homeostasis, impair mitochondrial bioenergetics and provoke a pro-inflammatory response in the brain, besides causing morphological alterations and death to neural cells. Furthermore, the observations of severe neurological symptoms and alterations of cerebral magnetic resonance imaging manifested mainly during crises of metabolic decompensation, characterized by excessive accumulation of BCAA and BCKA, suggest their relevant role on MSUD neuropathology. On the other hand, apart from their presumable acute involvement during crises, it is conceivable that chronic alterations of these neurochemical parameters may cumulatively contribute to the long-term neuropsychiatric morbidity of the affected patients. Current MSUD therapies based on dietary regimens and liver transplantation significantly improve peripheral BCAA biochemistry and significantly reduce mortality and metabolic crises. However, they still fail to prevent long-term neuropsychiatric symptoms in a considerable number of MSUD patients. Additional therapeutic approaches, aiming to regulate cellular redox status, mitochondrial functions, and neuroinflammation, including antioxidant and bioenergetics stimulating drugs targeting the

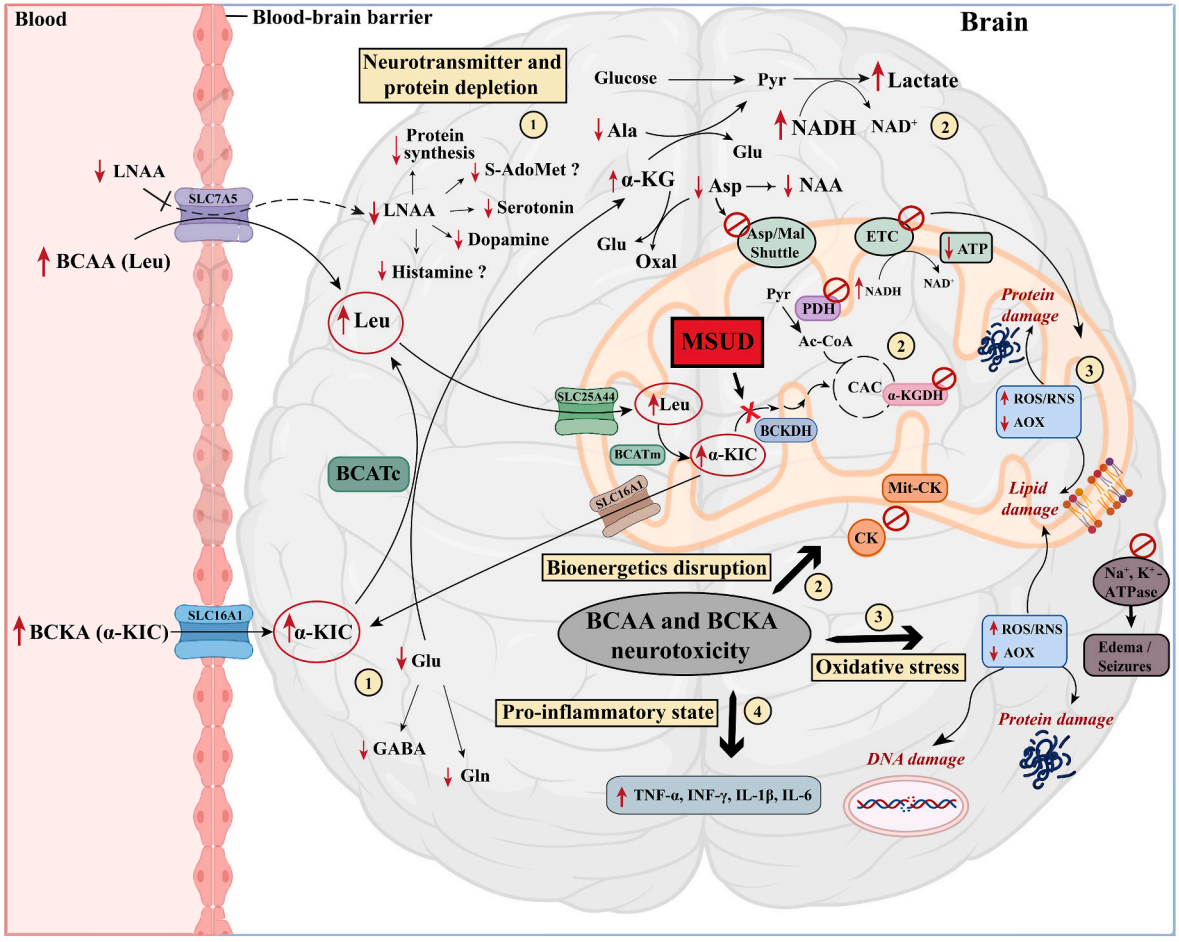

Fig. 3. Pathomechanisms of neurodegeneration in MSUD. Accumulation of the BCAA and BCKA, particularly Leu and α-KIC leads to **(1) Amino acid and neurotransmitter imbalance** - Increased plasma Leu concentrations impair cerebral LNAA uptake through the blood-brain barrier by competition, decreasing their availability for protein, dopamine, serotonin histamine and S-AdoMet synthesis. In addition, α-KIC accumulation in neuronal cytosol decreases Glu and increases α-KG levels by reverse transamination, also leading to a reduction of the Glu-derived Gln and GABA, as well as of Ala and Asp due to α-KG transamination. Pyruvate and excessive reducing equivalents generate high amounts of lactate. **(2) Bioenergetics disruption** - High cerebral concentrations of BCAA and BCKA inhibit the activities of CK, CAC and ETC, provoking impairment of ATP formation and NADH oxidation. Disruption of mitochondrial homeostasis associated with impaired aspartate/malate shuttle promote an increase of cytosolic NADH that accelerates lactate formation; **(3) Oxidative stress** – Brain accumulation of BCAA and BCKA induces ROS and RNS generation, oxidative damage to biomolecules and decreases the AOX defenses. **(4) Pro-inflammatory state induction** – Increased brain levels of BCAA and BCKA causes overproduction of the pro-inflammatory cytokines TNF-α, INF-γ, IL-1β and IL-6. Ac-CoA: acetyl-CoA; Ala: alanine; AOX: antioxidant; Asp: aspartate; BCAA: branched-chain amino acids; BCATc: cytosolic branched-chain aminotransferase; BCATm: mitochondrial branched-chain aminotransferase; BCKA: branched-chain α-keto acids; BCKDH: branched-chain α-keto acid dehydrogenase complex; CAC: citric acid cycle; CK: creatine kinase; ETC: electron transfer chain; GABA: γ-aminobutyric acid; Glu: glutamate; Gln: glutamine; IL-1β: interleukin-1β; IL-6: interleukin-6; INF-γ: interferon-γ; α-KG: α-ketoglutarate; α-KGDH: α-ketoglutarate dehydrogenase; α-KIC: α-ketoisocaproic acid; Leu: leucine; LNAA: large neutral amino acids; Mit-CK: mitochondrial creatine kinase; MSUD: maple syrup urine disease; NAA: N-acetylaspartate; Oxal: oxaloacetate; Pyr: pyruvate; PDH: pyruvate dehydrogenase; RNS: reactive nitrogen species; ROS: reactive oxygen species; S-AdoMet: S-adenosyl-methionine; TNF-α: tumour necrosis factor-α.

mitochondria, may hopefully become an important focus in the future to open novel avenues of drug development in order to ameliorate the neurological symptomatology improving disease progression.

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Author contributions

Alexandre Umpierrez Amaral constructed the figures and tables and wrote the manuscript; Moacir Wajner planned and wrote the manuscript.

Declarations of competing interest

None.

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