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Visions & Reflections

Methylmalonic acid – an endogenous toxin?

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Abstract. Methylmalonic acid was previously considered as major neurotoxin in methylmalonic acidurias. In contrast, recent studies support the notion that other metabolites deriving from propionyl-coenzyme A, inducing synergistic inhibition of mitochondrial energy metabolism, are more important than methylmalonic acid to understand the neuropathogenesis of this disease. However, it is not yet known whether methylmalonic acid is involved in the induction of other organ manifestations in this disease, such as chronic renal failure.

Key words. Methylmalonic aciduria; organic acids; metabolic stroke; excitotoxicity; energy metabolism; renal failure.

Methylmalonic acidurias are a group of inborn errors of metabolism that are biochemically characterized by an accumulation of methylmalonic acid (MMA). They are caused by an inherited deficiency of methylmalonyl-coenzyme A (CoA) mutase (MCM; EC 5.4.99.2) or by defects in the synthesis of 5′-deoxyadenosylcobalamin, the cofactor of MCM. Besides the name-giving organic acid MMA, also other organic acids deriving from propionate or propionyl-CoA accumulate, e.g. (2S,3S)-methylcitric acid (MCA) and 3-hydroxypropionic acid [1]. Although the etiology of methylmalonic acidurias is heterogenous, the clinical presentation of affected patients is quite similar. The disease course is complicated by acute metabolic crises which are precipitated by catabolic state, e.g. during intercurrent infectious diseases or prolonged fasting. These crises are characterized by lactic acidosis, hypoglycemia, hyperketonemia and hyperammonemia resulting in multiple organ failure and even death if untreated [1]. Despite continuous improvement of therapy during the last 20 years, the overall outcome of these patients remains disappointing, e.g. many patients still suffer from failure to thrive, psychomotor retardation, pancreatitis, renal failure and long-term neurological deficits [2–6].

'Metabolic stroke' and toxic organic acids

Acute extrapyramidal disease in these patients resulting from metabolic decompensation and, subsequently, bilateral destruction of the basal ganglia, in particular the globus pallidus, was considered to constitute a 'metabolic stroke' since hypoxemia or vascular insufficiency could be ruled out. It was hypothesized that metabolic stroke was induced by accumulating toxic organic acids [7]. A magnetic resonance (MR) study recently demonstrated restricted diffusion and elevated amounts of lactate and reduced *N*-acetylaspartate in the globus pallidus, signalling mitochondrial dysfunction and neuronal loss which confirmed the idea of metabolic stroke [8]. These findings have stimulated the search for candidate neurotoxins during the last years. The exact cerebral concentrations of MMA are not yet known. Since plasma concentrations are found in high micromolar to low millimolar concentrations and cerebrospinal fluid (CSF)

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concentrations equaled that of plasma concentrations in some patients [1, 9, 10], it was suggested that cerebral concentrations of MMA might be similar to plasma and CSF concentrations [10, 11], in particular during metabolic crisis. However, this is just a rough estimate and requires further investigation. Due to some structural similarities with the complex II (succinate dehydrogenase) inhibitors malonic and 3-nitropropionic acids MMA was suggested to exert properties similar to a mitochondrial toxin [11–13]. In fact, MMA induced striatal lesions and evoked rotational behavior and seizures in rats following stereotaxic administration of high local concentrations of MMA concentrations [14, 15]. Furthermore, MMA induced neuronal damage in dissociated neuronal cultures from rats at millimolar concentrations [16, 17]. The following mechanisms were considered relevant for MMA-induced neuronal damage: inhibition of (i) complex II [18], (ii) transmitochondrial malate shuttle [19], (iii) pyruvate carboxylase [10] and (iv) β-hydroxybutyrate dehydrogenase [18]. Furthermore, one study suggested cleavage of the methyl group of MMA by intracellular esterases to form the complex II inhibitor malonic acid [16]. Not surprinsingly, MMA was suggested to induce so-called secondary excitotoxicity [11], a concept originally established to describe the mechanisms involved in neuronal damage induced by malonic and 3-nitropropionic acids, involving mitochondrial dysfunction, increased generation of reactive oxygen species and increased vulnerability to glutamate-induced cell damage [12, 13].

Synergistic inhibition of mitochondrial energy metabolism – methylmalonic acids versus alternative toxic metabolites

The concept of MMA-induced neuronal damage, (partially) mimicking the inhibitory effects of malonic and 3-nitropropionic acids on mitochondrial respiratory chain, is a straightforward concept but has some shortcomings. Doubts have been raised whether MMA itself inhibits mitochondrial energy metabolism in a relevant way and whether MMA is the major neurotoxin in methylmalonic acidurias. In two recent studies from our laboratory, we clearly demonstrated that MMA (i) did not specifically inhibit respiratory chain complex II (or any other respiratory chain complex) in submitochondrial particles from bovine heart using spectrophotometric analysis and (ii) that MMA did not reduce the oxidation rate of ^{14}C-labelled pyruvate, malate, and succinate in 600 $\times$ *g* supernatants from C57Bl/6 mouse muscle [16, 20]. These results do virtually exclude any direct effect of MMA at pathophysiologically relevant concentrations on respiratory chain and mitochondrial transporter systems. However, the striking discrepancy between MMA inducing neuronal damage in vitro and in vivo and the lack of a direct inhibitory effect on mitochondrial respiratory chain and tricarboxylic acid cycle needed to be explained. For this purpose, we performed loading experiments in dissociated striatal cultures from rats, investigating the intracellular concentrations of accumulating organic acids by gas chromatography/mass spectrometry. By this approach, we could clearly show that incubation of cultured striatal rat neurons with millimolar concentrations of MMA induced an intracellular increase in MMA, MCA and – to a lesser extent – malonic acid in a time-dependent way [17]. An obvious shortcoming of MMA loading in cells with normal MCM actitivity is that accumulation of the proximal precursor propionyl-CoA and products of alternative propionyl-CoA oxidation – MCA and malonyl-CoA – necessitates a reversible flux through different enzymatic steps against the normal flux through this pathway. Reversibility of the propionyl-CoA pathway was recently demonstrated in liver and heart tissues of pigs [21]. In analogy to this study, further investigations using radiolabelled precursor metabolites are required to estimate the flux through alternative propionyl-CoA oxidation pathways in MCM-deficient cells, e.g. using recently generated *mut*$^{-/-}$ mice [22], and to investigate whether the complex II inhibitor malonic acid is formed from malonyl-CoA, an intermediate in this pathway. Alternatively, malonyl-CoA is also formed by carboxylation of acetyl-CoA catalyzed by acetyl-CoA carboxylase, the key enzyme in endogenous fatty acid synthesis. This reaction is facilitated by inhibition of the TCA cycle, resulting in an accumulation of acetyl-CoA, and a concomitant activation of acetyl-CoA carboxylase by MCA [23]. In contrast, previously suggested cleavage of the methyl group of intracellular esterases is quite unlikely to explain the formation of malonic acid from MMA [15]. Notably, we recently found increased urinary excretion of malonic acid (up to 150 mmol/mol creatinine) in *mut*° patients during metabolic decompensation [S. Kölker, unpublished observation]. However, the role of malonic acid in the neuropathogenesis of methylmalonic acidurias remains unclear.

Although the intracellular pathways which are involved in this scenario need careful evaluation, our findings shifted the focus of interest to the metabolites of alternative propionyl-CoA oxidation, hypothesizing that these compounds synergistically contribute to neuronal damage in methylmalonic acidurias rather than MMA alone. It was previously suggested that MCA was formed by condensation of propionyl-CoA and oxaloacetate and inhibited the first three enzymes of the tricarboxylic acid cycle, and the mitochondrial citrate transporter, resulting in mitochondrial accumulation of MCA [23]. Notably, a recent study demonstrated that MCA mainly contributed to propionate sensitivity of bacteria lacking the MCA cycle as detoxifying mechanism [24]. In addition to MCA and malonic acid, propionyl-CoA or propionic acid them-

selves were considered as neurotoxic agents, (i) inducing hyperammonemia by inhibition of *N*-acetylglutamate synthetase [25] and (ii) decreasing oxidative phosphorylation by inhibition of pyruvate dehydrogenase complex [26]. Since all single mechanisms of MCA, malonic acid and propionyl-CoA induce various inhibitory effects on respiratory chain, pyruvate dehydrogenase complex and tricarboxylic acid cycle, we have hypothesized that secondary excitotoxic damage of the brain results from a synergism of these effects [17, 20]. Obviously, further studies are required to investigate the involvement of the above-mentioned (and maybe additional) metabolites and their proposed synergistic interactions in greater detail than before to unravel the major neurotoxic principle in methylmalonic acidurias.

Methylmalonic acid and renal failure

Although it seems doubtful that MMA plays a major role as endogenous neurotoxin, it cannot be excluded that it might be involved in the induction of renal failure in methylmalonic acidurias. A systematic investigation of nephrotoxic mechanisms in methylmalonic acidurias, however, has not yet been performed. Therefore, considerations on this topic are at present quite speculative. Preliminary results in rats suggested that administration of MMA induced proteinuria and renal tubular injury [27]. Microscopy in these rats revealed that glomeruli appeared normal, whereas tubuli were dilated and tubulus epithelium showed mitochondrial swelling and disorganization of cristae. Although this study demonstrated some similarities with renal failure in methylmalonic acidurias considered to start with renal tubular dysfunction and tubulointerstitial nephritis [28, 29], it remains unclear whether this animal model is suitable for the investigation of MMA-induced renal failure [27].

Such investigations should also refer to the tissue-specific differences regarding the vulnerability to specific toxins and mechanisms. For example, an investigation of excitotoxic pathways might be important in neurons [17] but is obviously not relevant in kidneys. In contrast, an interesting approach to study nephrotoxic mechanisms in methylmalonic acidurias might be to investigate the effects of accumulating metabolites on tubular transporter systems, such as the dicarboxylic acid carrier (DCC). DCC is responsible for the transport of glutathione and dicarboxylic acids (e.g. MMA) into kidney mitochondria and is important for the protection of tubular cells against chemical-induced injury [30]. One could speculate that MMA-induced inhibition of glutathione transport via DCC in synergism with inhibition of TCA cycle and respiratory chain by MCA, propionyl-CoA and malonic acid could result in the induction of renal failure. This hypothesis could be easily tested in tubular cells by determining the rate of glutathione transport into intact mitochondria in the presence of accumulating metabolites. Notably, renal failure is usually found only in methylmalonic acidurias but not in propionic acidemia. Both diseases share similar neurologic abnormalities, showing a particular affection of the basal ganglia [2], and increased urinary excretion of metabolites from alternative oxidation of propionyl-CoA, whereas renal failure and increased urinary excretion of MMA are only found in methylmalonic acidurias. However, it remains to be elucidated whether the major clinical difference between these diseases, i.e. renal failure, is based on the major biochemical difference, i.e. accumulation of MMA, or is just coincidental.

In conclusion, the search for toxic organic acids has stimulated basic research on methylmalonic acidurias and other inborn errors of metabolism, such as glutaric aciduria type I [31], D-2-hydroxyglutaric aciduria [32, 33] and long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency [34], shifting the focus of interest in some pathognomonic accumulating organic acids from diagnostically relevant metabolites to pathophysiologically interesting endogenous toxins.

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