Pathophysiology of propionic and methylmalonic acidemias. Part 2: Treatment strategies

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Abstract
Despite realizing increased survival rates for propionic acidemia (PA) and methylmalonic acidemia (MMA) patients, the current therapeutic regimen is inadequate for preventing or treating the devastating complications that still can occur. The elucidation of pathophysiology of these complications allows us to evaluate and rethink treatment strategies. In this review we display and discuss potential therapy targets and we give a systematic overview on current, experimental and unexplored treatment strategies in order to provide insight in what we have to offer PA and MMA patients, now and in the future. Evidence on the effectiveness of treatment strategies is often scarce, since none were tested in randomized clinical trials. This raises concerns, since even the current consensus on best practice treatment for PA and MMA is not without controversy. To attain substantial improvements in overall outcome, gene, mRNA or enzyme replacement therapy is most promising since permanent reduction of toxic metabolites allows for a less strict therapeutic regime. Hereby, both mitochondrial-associated and therapy induced complications can theoretically be prevented. However, the road from bench to bedside is long, as it is challenging to design a drug that is delivered to the mitochondria of all tissues that require enzymatic activity, including the brain, without inducing any off-target effects. To improve survival rate and quality of life of PA and MMA patients, there is a need for systematic (re)-evaluation of accepted and potential treatment strategies, so that we can better determine who will benefit when and how from which treatment strategy.

KEYWORDS
methylmalonic acidemia, pathophysiology, propionic acidemia, treatment strategies

1 | INTRODUCTION

Whereas only a few decades ago patients with propionic acidemia (PA) and methylmalonic acidemia (MMA) had a very poor life expectancy, patients now tend to reach adulthood.
Next to general advances in clinical care, this has been accomplished through the implementation of a protein-restricted diet, carnitine supplementation and the supplementation of the cofactor cobalamin in MMA. These therapies were conceived when pathophysiological mechanisms at play in these diseases were recognized. However, PA and MMA patients are still greatly at risk for developing debilitating complications, and the overall outcome remains unsatisfying. The currently available therapies are inadequate for prevention or treatment of these complications. Despite extensive research, no new therapies have been implemented over the past decade, while there is an urgent need for improved treatment.

In the coming decade, the tide may turn. Pathophysiology of complications in PA and MMA is studied more and more and thereby gradually elucidated (reviewed in Haijes et al5). Improved understanding of pathophysiology allows us to evaluate and rethink our treatment strategies. In this review we summarize the biochemical consequences of PA and MMA to display potential therapy targets and we provide a systematic overview on current, experimental and unexplored treatment strategies in PA and MMA in order to provide insight in what we have to offer PA and MMA patients, now and in the future.

2 | METABOLITE ACCUMULATION IN PA AND MMA

The genes harboring mutations that give rise to PA and MMA all encode mitochondrial enzymes. PA is caused by a deficiency of propionyl-CoA carboxylase (PCC, EC 6.4.1.3), a protein complex of which the two subunits are encoded by PCCA and PCCB. Isolated methylmalonic acidemia (MMA) is either caused either by a deficiency of methylmalonyl-CoA mutase (MCM, EC 5.4.99.2, encoded by MUT), by diminished synthesis or availability of its cofactor 5'-deoxyadenosylcobalamin which is associated with cobalamin A, B or D deficiency (encoded by MMAA, MMAB or MMADHC, respectively), or by a deficiency of methylmalonyl-CoA epimerase (MCE, EC 5.1.99.1, encoded by MCEE). The protein encoded by MMAA is reported to act as a reactivating factor for MCM by promoting the replacement of the inactive cofactor hydroxycoabalin by the active cofactor adenosylcobalamin. \(^6\) MMAB encodes the adenosyltransferase (EC 2.5.1.17) that converts cobalamin into adenosylcobalamin and the protein encoded by MMADHC is proposed to act as a trafficking chaperone that delivers processed cobalamin to its target enzymes, including MCM. \(^7\) The biochemical pathway harboring PCC, MCM, MCE, and adenosylcobalamin is illustrated in Figure 1.

Within isolated MMA, the cause and range of accumulation of toxic metabolites, the natural history and the prognosis differ between the different types. \(^8\)-10 MMA caused by deficiency of MCM is often distinguished in cobalamin unresponsive patients (mut\(^{-}\)) and cobalamin responsive patients (mut\(^{+}\)). Cobalamin A deficient patients are usually cobalamin responsive, cobalamin B deficient patients can either be cobalamin unresponsive or responsive. Mut\(^{0}\) and cobalamin B deficient patients are considered severely affected regarding morbidity and mortality, whereas mut\(^{-}\) and cobalamin A deficient patients are considered to present with a milder form of MMA. Cobalamin D deficiency can either result in combined methylmalonic aciduria and homocystinuria, in isolated homocystinuria (variant 1) or in isolated MMA (variant 2). This type of isolated MMA is very rare compared to MCM deficiency and cobalamin A and B deficiency, and results in a cobalamin responsive phenotype similar to mut\(^{-}\) and cobalamin A deficiency. \(^11\) MCE deficiency is also very rare and its' natural history is very variable, with asymptomatic patients, patients presenting with acute metabolic decompensations and patients having also a sepiapterin reductase deficiency. \(^12\) Despite the differences between the different forms of isolated MMA, many publications assemble research data on MCM, cobalamin A and cobalamin B deficiency, and discuss isolated MMA as one disease entity. Therefore, in this review we consider isolated MMA as one disease entity as well, but discuss the different types of MMA separately whenever possible.

The enzymes deficient in PA and MMA have an indispensible role in the breakdown of the branched-chain amino acids valine and isoleucine, and threonine and methionine. In addition, they act in the catabolism of propionyl-CoA that is formed in anaerobic fermentation of carbohydrates by gut bacteria and in the breakdown of odd-chain fatty acids and cholesterol side chains (Figure 1). The shares of each of these three supply routes are approximately 50%-25%-25%. \(^13\), \(^14\) Blockade of the conversion of propionyl-CoA into methylmalonyl-CoA and succinyl-CoA results in accumulation of the toxic metabolite propionyl-CoA. Propionyl-CoA is converted through glycine N-methylacyltransferase into the nontoxic metabolite propionylglycine and through carnitine acetyltransferase into the—presumably—nontoxic metabolite propionylcarnitine. However, propionyl-CoA is also converted into propionic acid, hydroxypropionic acid and through citrate synthase into 2-methylcitrate, all toxic metabolites. In addition, propionyl-CoA—as competitive inhibitor of N-acetylglutamate synthase—inhibits N-acetylglutamate synthesis, resulting in a lack of stimulation of carbamoylphosphate synthase. As a consequence, detoxification of ammonia by the urea cycle is decreased, resulting in increased ammonia levels. \(^15\) In MMA patients, accumulation of methylmalonyl-CoA results in accumulation the toxic metabolite methylmalonic acid and often in accumulation of methylmalonylcarnitine (Figure 1). The extent to which
FIGURE 1  Legend on next page.
methylmalonic acid is increased in MMA patients differs between the different subtypes. Fowler et al reported that urinary concentrations of methylmalonic acid are between 5000 and more than 10 000 mmol/mol creatinine for mut0 and cobalamin B deficient patients, whereas mut− and cobalamin A deficient patients have methylmalonic acid urinary concentrations between 1000 and 9000 mmol/mol creatinine. This range in cobalamin D deficient patients is between 500 and 10 000, and in MCE deficient patients the concentrations are between 100 and 1000 mmol/mol creatinine, demonstrating important differences in severity of the metabolic defect, and thus, expected toxicity.

3 | EFFECTS OF ACCUMULATING TOXIC METABOLITES

In PA patients the toxic metabolites propionyl-CoA, 2-methylcitric acid, ammonia, propionic acid and 3-hydroxypropionic acid accumulate. In MMA patients, also methylmalonyl-CoA and methylmalonic acid are increased. Presumed effects of these metabolites are illustrated in Figure 2.

3.1 | Propionyl-CoA

Propionyl-CoA induces secondary deficiencies of a range of mitochondrial enzymes. In rat liver mitochondria, it was demonstrated that propionyl-CoA is a competitive inhibitor of N-acetylglutamate synthetase. Propionyl-CoA is a substrate for this enzyme and is converted into N-propionylglutamate, which is a competitive inhibitor of carbamoylphosphate synthase. These inhibitions result in a secondary deficiency of the urea cycle and hyperammonemia. It has been suggested that propionyl-CoA, by inhibiting urea synthesis, also prevents the consumption of NADPH which will inhibit the glycine cleavage system. This mechanism may explain the increase of glycine in body fluids of PA and MMA patients, although it has not been confirmed in other studies. Bremer demonstrated that propionyl-CoA (as well as acetyl-CoA) inhibits activity of soluble pyruvate dehydrogenase from pig heart and kidney and suggests that inhibition occurs through competition of propionyl-CoA with CoA. Gregersen confirmed the findings of Bremer and the suggested competition of propionyl-CoA with CoA in pig kidney tissue, but adds that inhibition of succinyl-CoA synthetase activity could also contribute to reduced oxidation of pyruvate, as suggested by Stumpe et al.

3.2 | 2-Methylcitric acid

In rat liver mitochondria, 2-methylcitric acid was demonstrated to compromise the citric acid cycle by inhibition of citrate synthase, aconitase and isocitrate dehydrogenase. It was also shown that 2-methylcitric acid competes with citrate for the mitochondrial tricarboxylate carrier that exports citrate from mitochondria into cytosol. In 3D organotypic rat brain cell cultures, Jafari et al demonstrated that exposure to 2-methylcitric acid, even in low concentrations, resulted in a significant decrease in astrocyte fiber density, an enlargement of astrocytic bodies and a swelling of proximal fibers, whereas treatment with propionic or methylmalonic acid did not show any significant changes compared to controls. In addition, it was demonstrated that 2-methylcitric acid exposure resulted in diminished axonal outgrowth, retarded neuronal differentiation or axonal degeneration, and retarded differentiation of oligodendrocytes. Biochemically, 2-methylcitric acid led to an increase in ammonia levels and a decrease in glutamine levels in the medium of the brain cells. In a subsequent study, all effects of 2-methylcitric acid reported by Jafari et al were confirmed. Moreover, it was demonstrated that the earliest event was ammonium accumulation, occurring even after exposure to a single dose of 2-methylcitric acid. The authors suggest that ammonia accumulation may be the first pathogenic event that triggers further cellular and metabolic responses. This might imply that an increase of 2-methylcitric acid above a critical threshold, even for a short duration, might induce brain damage.

FIGURE 1 Metabolite accumulation in PA and MMA, and associated therapy targets. Metabolites are depicted in light gray rectangles, toxic metabolites are depicted in red rectangles. Enzymes are depicted in light gray ovals. Cofactors for enzymes are depicted in white ovals. Primary supply routes of the propionate pathway are depicted in dark gray rectangles. Increased concentrations of metabolites are depicted by the larger red arrows, decreased enzyme activities are depicted by the smaller blue arrows. Targets for current and experimental treatment strategies are depicted by green circles, the numbers correspond to the numbers of the therapy targets (TT) in Table 1 and throughout the text.
observed an increase in the number of apoptotic cells in brain cell cultures exposed to 2-methylcitric acid, making 2-methylcitrate an important pathogenic player in PA and MMA.

Cudrē-Cung et al further demonstrated that 2-methylcitric acid led to a 40% decrease of glutamine synthetase activity, affecting ammonia detoxification in brain cells. They observed a decrease of extracellular glutamine levels, but an increase of intracellular glutamine levels, implicating that brain cells may be exposed to higher glutamine concentrations than predicted by blood glutamine levels.

Whereas Amaral et al could not confirm an altered glutamine synthetase activity; they demonstrated that 2-methylcitric acid inhibited glutamate dehydrogenase activity towards the formation of α-ketoglutarate in rat brain mitochondria, through competition with glutamate. This affects the availability of α-ketoglutarate for proper functioning of the citric acid cycle. In addition, Amaral et al...

**FIGURE 2** Effects of toxic metabolites in PA and MMA, and associated therapy targets. Metabolites are depicted in light gray rectangles, toxic metabolites are depicted in red rectangles. Enzymes are depicted in light gray ovals. Cofactors for enzymes are depicted in white ovals. Cellular processes are depicted in dark gray rectangles. Increased concentrations of metabolites are depicted by the larger red arrows, increased enzyme activities are depicted by smaller red arrows. Decreased concentrations of metabolites are depicted by the larger blue arrows, decreased enzyme activities are depicted by the smaller blue arrows. Targets for current or experimental treatment strategies are depicted by green circles, the numbers correspond to the numbers of the therapy targets (TT) in Table 1 and throughout the text. Asterisks indicate the effects of toxic metabolites that are expected to be present in MMA patients, but not in PA patients, according to the available literature.
demonstrated that 2-methylcitric acid induced mitochondrial permeability transition pore opening, that ultimately led to mitochondrial energy dysfunction, as illustrated by decreased ATP synthesis.

In summary, 2-methylcitric acid leads to hyperammonemia and the risk for brain cell apoptosis and it decreases both enzymatic activities and substrate availability of the citric acid cycle, thereby leading to mitochondrial energy impairment.

3.3 | Ammonia

The toxic effects of ammonia accumulation in brain were reviewed by Braissant et al. Ammonia accumulation in patients with urea cycle disorders, is glutamine increase through activation of glutamine synthetase, which is expected to be the cause of the observed astrocyte swelling. In contrast to patients with urea cycle defects, PA and MMA patients have normal or even decreased concentrations of glutamine in plasma during acute metabolic decompensation.

Ammonia accumulation also activates the Na\(^+\)-K\(^+\)-Cl\(^-\) cotransporter, increasing water entry into astrocytes. This might explain the observed astrocyte swelling in normoglutaminergic hyperammonemia. In astrocyte cultures exposed to ammonia, synthesis of nitric oxide (NO) is stimulated. These cultures show opening of the mitochondrial permeability transition pore, which leads to altered oxidative phosphorylation, diminished ATP synthesis, production of reactive oxygen species (ROS) and cell death. It is expected that these effects are the result of the interaction between NO and superoxide anions, leading to the formation of highly toxic peroxynitrites. This might also contribute to altered permeability of the blood-brain barrier, thereby contributing to vasogenic edema, which is often observed in patients with hyperammonemia.

3.4 | Propionic acid

Propionic acid is considered a toxic metabolite since in the brain of rats exposed to concentrations of propionic acid that simulate concentrations in PA patients, a 30% inhibition of Na\(^+\)/K\(^+\) ATPase was demonstrated, as well as an increase of lipid and protein peroxidation and a decrease of antioxidant potential, a reduction of ganglioside species, increased phosphorylation of cytoskeletal proteins possibly via N-methyl-D-aspartate (NMDA) receptor activation, an induction of long-term behavioral and cognitive deficits and an induction of convulsions possibly via NMDA receptor activation. In peripheral leukocytes incubated with propionic acid, it was demonstrated that propionic acid induces DNA damage, possibly via formation of free radicals.

3.5 | 3-Hydroxypropionic acid

Little is known on the toxic effects of 3-hydroxypropionic acid. There is one report, demonstrating that 3-hydroxypropionic causes increases of CoA esters and thereby overloading of the citric acid cycle and inducing proteolysis.

3.6 | Methylmalonyl-CoA

In MMA patients, methylmalonyl-CoA is increased. Methylmalonyl-CoA is reported to be a mild inhibitor of N-acetylglutamate synthetase and an inhibitor of carbamoylphosphate synthase, thereby inhibiting the urea cycle. In addition, methylmalonyl-CoA has been demonstrated to inhibit pyruvate dehydrogenase activity in rat hepatocytes.

3.7 | Methylmalonic acid

In MMA patients, also the toxic metabolite methylmalonic acid is increased. In brains and livers of rats exposed to concentrations of methylmalonic acid similar to those in MMA patients, a decreased activity of \(\beta\)-hydroxybutyrate dehydrogenase was observed, as well as a decreased activity of Na\(^+\)/K\(^+\) ATPase, mitochondrial creatine kinase, the respiratory chain complexes I-III, a decreased transport of succinate through membranes and a decreased activity of glutamate decarboxylase which negatively correlated with the duration of methylmalonic acid-induced seizures, that possibly occurred through the activation of NMDA receptor activation. Melo et al confirmed the finding of a methylmalonic acid-induced decreased activity of glutamate decarboxylase in rat brain mitochondria, but this inhibition was only minor, whereas \(\alpha\)-ketoglutarate dehydrogenase was more profoundly inhibited by methylmalonic acid. Moreover, the exchange of methylmalonic acid for \(\alpha\)-ketoglutarate seems to deplete mitochondria from \(\alpha\)-ketoglutarate, but this could not be confirmed in vivo. In addition, a methylmalonic acid-induced increase of lipid and protein peroxidation and decrease of superoxide dismutase activity was observed in rat brain and kidney tissue, which was further amplified by the induction of renal failure.

3.8 | Simultaneous and synergistic toxicity

The various effects of the toxic metabolites occur simultaneously and are synergistic. Altogether, based on the best available evidence from studies performed on the consequences of accumulation of the toxic metabolites in PA and MMA, one could expect in PA and MMA patient tissues decreased activities of N-acetylglutamate synthetase, carbamoylphosphate synthase, pyruvate dehydrogenase, citrate synthase, aconitase, isocitrate dehydrogenase, \(\alpha\)-ketoglutarate dehydrogenase,
succinyl-CoA synthetase, respiratory chain complex III, glutamate dehydrogenase and the glycine cleavage system and an increased activity of lactate dehydrogenase. In addition, one could expect in PA patient tissues a decrease in glucose concentrations and an increase in lactate and ammonia concentrations, as well as the formation of NO, ROS, peroxynitrates, and peroxidized lipids and proteins, the induction of DNA damage, reduced anti-oxidant potential and diminished ATP synthesis (Figure 2). In MMA patients, but not in PA patients, one could additionally expect decreased activities of β-hydroxybutyrate dehydrogenase, creatine kinase, glutamate decarboxylase and respiratory chain complex I and II (Figure 2, marked with an asterisk).

The presented evidence, despite being the best available, should be interpreted with caution, since nearly all studies were performed in vitro, mostly on mammalian—but not human—cells. It is not clear to what extent the conditions in the experiments are similar to physiological conditions in PA and MMA patients, and whether the concentrations of toxic metabolites that animals or cell lines were exposed to reflect their in vivo concentrations. Furthermore, exposure of cells to metabolites, requiring uptake and accumulation to reflect the disease state fundamentally differs from the true disease situation in which cells endogenously accumulate these metabolites. Moreover, especially in the studies covering the toxic effects of propionic acid and methylmalonic acid, the concentrations of the other potentially toxic metabolites were not measured. Therefore, it is not clear whether the observed effects are the direct result of propionic or methylmalonic acid, or indirect and resulting from secondary metabolites like propionyl-CoA, methylcitric acid or any other toxic metabolite. Thus, it cannot be concluded that the toxic effects attributed to methylmalonic acid, that might be specific for MMA patients, are not present in PA patients.

While these limitations are critical for the interpretation of the presented data, it can still be concluded that the combined results of all changes induced by toxic metabolites formed in PA and MMA, are mitochondrial energetic failure and increase of ROS formation leading to oxidative stress.

4 | THERAPY TARGETS AND TREATMENT STRATEGIES

Each step in the biochemical cascade, as illustrated in Figures 1 and 2, ultimately contributing to mitochondrial energetic failure and oxidative stress, is a potential target for treatment. We discern two main treatment strategies. The first strategy is aimed to reduce the amount of accumulating toxic metabolites. This aim can be attained by decreasing supply, improving enzyme activity or increasing disposal. Therapeutic targets for this treatment strategy are displayed in Figure 1 and listed in Table 1. The second treatment strategy is aimed to prevent or treat mitochondrial energetic failure and to prevent or treat the increase of ROS formation. Therapy targets for this treatment strategy are displayed in Figure 2 and also listed in Table 1. We will discuss the current, experimental and unexplored treatment strategies in PA and MMA (Table 1).

4.1 | Treatment strategy 1: reduce the amount of toxic metabolites

4.1.1 | Current treatment strategies

To lessen the most important supply of toxic metabolites, patients follow a life-long protein restricted diet, with limited amounts of isoleucine, valine, threonine, and methionine1 (Figure 1 and Table 1, therapy target number 1 (TT#1)). In addition, to reverse catabolism, a high caloric diet is provided, in acute situations often with i.v. glucose.51 To tackle the second source of toxic metabolites, antibiotics (particularly metronidazole) are prescribed to diminish propionyl-CoA from gut bacteria through selective bowel decontamination.14,52 Laxatives, stimulating the intestinal flow in constipated patients, also aim to reduce propionyl-CoA from gut bacteria53 (TT#2).

To stimulate residual enzyme activity, hydroxycobalamin is prescribed to cobalamin responsive MMA patients: mut−, cobalamin A and cobalamin D deficient patients2 (TT#4). In very severely affected patients, solid organ transplantation is considered (liver transplantation in PA and MMA, or liver-kidney transplantation in MMA), since normal activity of the enzyme in the donor organ can (partly) compensate for the patients’ enzyme deficiency54,55 (TT#3). Kidney transplantation is also performed, solely in MMA patients, in order to treat chronic kidney disease. The primary intent is not to provide additional enzymatic activity, although it might serve as such.106

To increase disposal of toxic metabolites, L-carnitine is prescribed, which stimulates conversion of propionyl-CoA into the nontoxic propionylcarnitine, and in limited amounts in MMA patients into methylmalonylcarnitine4 (TT#7). Supplementation of L-carnitine restores intracellular carnitine levels and thereby it restores the impaired fatty acid oxidation due to the relative carnitine deficiency.107 During acute or chronic metabolic decompensations, when ammonia accumulates, N-carbamylglutamate is prescribed to stimulate carbamoylphosphate synthase thereby optimizing detoxification of ammonia by the urea cycle80,81 (TT#5). Additionally, the ammonia scavenger sodium benzoate is used, which acts by promoting conjugation with glycine to form hippuric acid82 (TT#6). Another possibility to reduce ammonia levels in acute phases is extracorporeal detoxification, in infants via continuous venous-venous hemodiafiltration and in adults either via continuous venous-venous hemodiafiltration.
| Treatment strategy | Substrategy | Therapy target (TT) | Treatment | Current status | References |
|--------------------|-------------|---------------------|-----------|----------------|------------|
| Reduce amount of toxic metabolites | Reduce supply | Figure 1 #1 | Isoleucine, valine, threonine and methionine restriction (with or without amino acid supplementation) | In clinical guideline | 1,49,50 |
| | | Figure 1 #1 | High caloric diet during illness | In clinical guideline | 49,51 |
| | | Figure 1 #2 | Antibiotics | In clinical guideline | 14,49,52 |
| | | Figure 1 #2 | Laxatives | In clinical guideline | 49,53 |
| | | | Reduce odd-chain fatty acids and cholesterol | In clinical guideline | |
| Increase enzyme activity | | Figure 1 #4 | Hydroxycobalamin | In clinical guideline for cobalamin-responsive MMA patients | 2,49 |
| | | Figure 1 #3 | Solid organ transplantation (liver or combined liver-kidney transplantation) | In clinical guideline; advice to consider in severely affected patients | 49,54,55 |
| | | Figure 1 #3 | Gene therapy | Experimental in animal models | 56-70 |
| | | Figure 1 #3 | Promotion of premature stop codon read-through | Experimental in cell lines | 71-73 |
| | | Figure 1 #3 | Messenger RNA therapy | Experimental in animal models | 74 |
| | | Figure 1 #3 | Pharmacological stabilization of enzyme activity | Experimental in cell lines | 75 |
| | | Figure 1 #3 | Enzyme replacement therapy | Experimental in animal models | 76-78 |
| | | Figure 1 #3 | Hepatocyte transplantation | Experimental in animal models | 79 |
| Increase disposal | | Figure 1 #5 | N-Carbamylglutamate | In clinical guideline | 49,80,81 |
| | | Figure 1 #6 | Sodium benzoate | In clinical guideline | 49,82 |
| | | Figure 1 #6 | Extracorporeal detoxification | In clinical guideline | 49,51 |
| | | Figure 1 #7 | l-Carnitine | In clinical guideline | 4,49 |
| | | Figure 1 #8 | Sodium bicarbonate | In clinical guideline | 49 |
| | | | Induce N-acetyltransferase activity | Unexplored | |
| | | | Scavenger for methylmalonic acid, propionic acid, 3-hydroxypropionic acid or 2-methylcitric acid | Unexplored | |
| Prevent or treat mitochondrial energetic failure and increase of ROS formation | Energetic supply | Figure 2 #8 | Succinate | Experimental in animal models | 83,84 |
| | | Figure 2 #9 | Citric acid | Experimental in patients | 85 |
| | | Figure 2 #10 | Creatine | Experimental in animal models | 83,84 |
| | | | Fumarate, malate, alpha-ketoglutarate | Unexplored | |
| | | | Glutamate | Unexplored | |
Sodium bicarbonate (TT #8) is used to reduce the acidic load of all the formed organic acids. In addition, it might induce forced diuresis and alkalinization of urine, aiding renal clearance of methylmalonic acid in MMA patients. Each of these treatment strategies are applied in PA and MMA patients, are discussed in the most recent and most comprehensive guideline and are considered to be best practice (Table 1). However, none of these treatment strategies have been tested in randomized clinical trials since it is unethical to test an intervention that is considered beneficial (and thereby withholding the intervention from some patients) and since conducting a randomized clinical trial in rare inborn errors of metabolism is very challenging (Ah). Hence, clinical evidence supporting the currently implemented treatment strategies is limited. We will give three examples of treatment strategies that have raised controversy and that could individually be emphasized in a full review. First, since the identification of PA and MMA as inborn errors of metabolism, there has been controversy about the added value of amino acid-based formulas in the dietary treatment of PA and MMA patients. This debate is based on the relatively high amount of leucine in these formulas that might induce deficiencies of isoleucine and valine. Still, in many metabolic clinics the use of amino acid-based formulas is common practice. Second, solid organ transplantation (liver or combined liver-kidney transplantation) is performed in metabolically unstable patients to provide additional enzymatic activity. The most recently reported outcomes are promising, but it remains questionable who to transplant, and when. In addition, since transplantation is not the only available treatment option, nor a curative treatment, the utility of performing solid organ transplantations in PA and MMA is questioned because of the scarcity of available donor organs. Decision-making for individual patients would benefit from a world-wide registry, enabling larger cohort studies into long-term benefits, risks, success rates and complications. Third, there is controversy about the use of N-carbamylglutamate in acute and chronic hyperammonemia. While acute amelioration of hyperammonemia due to N-carbamylglutamate is unequivocal, and no serious side effects due to N-carbamylglutamate are noted, other toxic metabolites do not decrease concurrently with ammonia. Since ammonia is the main biochemical parameter indicating acute metabolic decompensation, there is a risk of not recognizing and hence not managing (impending) acute metabolic decompensations adequately. It is surprising that N-carbamylglutamate is already recommended for treatment of acute management of hyperammonemia, while there is no

| Treatment strategy | Substrategy | Therapy target (TT) | Treatment | Current status | References |
|--------------------|-------------|---------------------|-----------|---------------|------------|
| Cofactor suppletion | Figure 2 #11 | Pyridoxine (vitamin B6) | Experimental in animal models | 45 |
| Anti-oxidants | Figure 2 #12 | Thiamine (vitamin B1) | Experimental in patients with lactic acidosis | 86,87 |
| | Figure 2 #13 | Coenzyme Q10 | Experimental in patients | 88-90 |
| | Figure 2 #14 | Ascorbic acid (vitamin C) | Experimental in animal models and in patients with lactic acidosis | 33,91-94 |
| | Figure 2 #14 | Alpha-tocopherol (vitamin E) | Experimental in animal models and in patients with optic atrophy | 47,91,92,95-97 |
| | Figure 2 #14 | CB agonist WIN, S-allylcysteine, gluthathione, GM1 ganglioside, melatonin, mitoQ, resveratrol, tiron, trolox. | Experimental in animal models | 42,47,98-102 |
| Electron transfer mediator | Figure 2 #14 | Uric acid, lipoic acid, retinol (vitamin A), β-carotene | Unexplored | |
| NMDA-receptor antagonists | Celecoxib, fish oil, kynurenic acid, MK-801 | Experimental in animal models | 37,45,47,83,84, 98,99,104,105 |

Abbreviations: NMDA, N-methyl-D-aspartate; ROS, reactive oxygen species.
data available on the long-term neurological outcomes of these patients. Efforts to obtain this data have unfortunately failed (Ah109), but the added value of N-carbamylglutamate in acute and chronic hyperammonemia on long-term neurological outcomes can only be substantiated by cohort studies investigating these outcomes.

### 4.1.2 | Experimental treatment strategies

Experimental strategies aiming to reduce the amount of accumulating toxic metabolites mainly focus on improving enzyme activity, either by gene therapy, supplementation of mRNA encoding the defective enzyme, enzyme replacement therapy or hepatocyte transplantation (TT#3).

In 1992, gene transfer using a retroviral vector resulted in correction of the metabolic defect in fibroblasts of patients with MMA.56 Next, short-term overexpression of MCM was demonstrated after intravenous administration of plasmids expressing recombinant Mut using asialoglycoprotein/polyllysine/DNA targeted delivery to the livers of healthy mice.57 Chandler et al58 demonstrated in both fetal murine fibroblasts and hepatocytes of two MMA patients that Mut gene delivery using a recombinant adenoviral vector was feasible, and resulted in correction of the enzymatic defect, suggesting that hepatocyte-directed gene delivery may be an effective treatment strategy. Subsequent studies in a Mut−/− mouse model demonstrated that gene therapy is a promising treatment option for MMA. Whereas intramuscular injection of adenovirus carrying the Mut gene was not effective, intrahepatic injection resulted in rescue of neonatal lethality.59 Intrahepatic injection with recombinant adenovirus-associated virus (rAAV) serotype 8 resulted in improved survival of the mice beyond one year, although there was still a gradual loss of transgene expression.60 Carillo-Carrasco et al61 demonstrated that the gene delivery in mice resulted in long-term phenotypic correction, but, in line with solid organ transplantation, it did not provide curative treatment. Senac et al62 tested rAAV serotype 9, as this serotype might be superior in tropism for both liver and kidney tissues, and demonstrated that gene delivery by this serotype also resulted in improved survival, using a 10-fold lower dose than used with rAAV serotype 8 vectors. In order to work towards clinical testing, efficacy and dosing of the rAAV serotype 8 vector were tested. In addition, the finding that AAV antibody titers are low in 21/24 MMA patients is encouraging and supports further development of systemic gene delivery as treatment for severely affected MMA patients.64 However, concerns have been raised about the safety of using rAAV vectors, since a dose-dependent increase in hepatocellular carcinoma is seen in Mut−/− mice treated with intrahepatic injection of rAAV vectors.67

Wong et al65 demonstrated that intravenous injection of a lentiviral vector in Mut−/− mice could also result in effective Mut gene transfer, as significant but incomplete biochemical correction of the phenotype was observed.

Gene therapy for PA was studied in a Pcca−/− mouse model. Both adenoviral and AAV serotype 8 vectors66 carrying Pcca were tested, and were administered via intraperitoneal injection. It resulted in an increased lifespan from 36 hours to 70 hours, indicating only a transient effect.66 A subsequent study using AAV serotype 8 vector delivered via intrahepatic injection resulted in a rescue of neonatal lethality in 64% of the mice and amelioration of the biochemical phenotype.67 These findings were confirmed by Guenzel et al,68 who generated a Pcca−/− mouse model that was partially rescued using a transgene, resulting in 2% residual enzymatic activity. These mice survived into adulthood and demonstrated the PA phenotype. It was demonstrated that adenovirus serotype 5 resulted only in a transient effect, whereas systemic administration of AAV serotype 8 resulted in more persistent correction of the biochemical phenotype.68 Next, the effectiveness of systemic therapy with AAV serotype 1 (muscle tropism), serotype 8 (liver tropism) and serotype rh10 (broad tropism) was studied and revealed that AAV serotype 8 in combination with a transthyretin promotor resulted in the most promising correction of the biochemical phenotype.69 The authors assume that gene therapy concurrently directed to hepatocytes and muscle cells would result in even better correction, as all tissues require PCC activity. They suggest that treatment of a wide array of tissues would represent the best option for PA disease correction.69 In a subsequent study, it was demonstrated that AAV-mediated expression of Pcca resulted in long-term phenotypic correction, although transgene expression decreased in the liver and in skeletal muscle. Surprisingly, tissue-specific loss of expression was only present in females.70 Next to gene therapy, there are more efforts to increase enzyme activity of PA and MMA patients. For patients harboring nonsense mutations, some drugs may promote premature termination codon read-through. For MMA patients, two studies were reported that achieved a slight increase in MCM activity in vitro.71,79 For PA patients, restoration of in vitro PCC activity to a level of 10%-15% was demonstrated.73 Though, protein function can only be restored in a fraction of patient cells with nonsense mutations, there is no information regarding the in vivo effectiveness and the clinical relevance of the attained effects is unclear.

An et al74 demonstrate that for MMA, intravenous administration of mRNA encoding Mut using a biodegradable lipid nanoparticle, results in a 75% reduction of plasma methylmalonic acid and increased MCM activity in the liver of Mut−/− mice, and that repeated dosing led to weight gain...
and improved survival. To the best of our knowledge, no mRNA treatment for PA has been reported.

Jorge-Finnigan et al reported that some pharmacological chaperones may stabilize the enzyme adenosyltransferase (encoded by *MMAB*) in patients with missense mutations in *MMAB* that destabilize the enzyme. They demonstrate that co-administration of some pharmacological chaperones and cobalamin resulted in increased adenosyltransferase stability in both *MMAB* patient fibroblasts and wild-type mice. 75

Enzyme replacement therapies have been studied in both MMA and PA. For PA, in both PCCA-defective and PCCB-defective lymphoblasts it was demonstrated that PCC sub-units could effectively be delivered to the mitochondria, using cell penetrating peptides and mitochondria targeting sequences, resulting in an apparent improvement of PCC activity. 77 A single intraperitoneal injection of PCC in a PCC defective mouse model, directed towards mitochondria, resulted in a substantial improvement of the biochemical phenotype. 78 Similarly, for MMA, in both patient fibroblasts and in CRISPR/Cas9-engineered HepG2 MUT−/− liver cells it was demonstrated that MCM could be effectively delivered to the mitochondria, enhancing cell viability, reducing methylmalonic acid levels, and enhancing albumin and urea secretion in the liver cells. 76

As an alternative for solid organ transplantation, to bypass the donor organ shortage, hepatocyte transplantation has been performed in—amongst others—urea cycle disorders in order to postpone liver transplantation.121-124 For MMA, transplantation of fetal liver cells in a *Mut−/−* mouse model attained donor cell engraftment in the liver, bone marrow and spleen, resulting in some correction of the biochemical phenotype. To realize greater disease correction, higher levels of engraftment are required. 79 Limitations of hepatocyte transplantation however still comprise limited supply of (good quality) donor livers, insufficient engraftment of hepatocytes and allogeneic rejection 125 Moreover, in line with solid organ transplantation, hepatocyte transplantation would most likely still not be a curative treatment.

Aiming to prevent any residual disease requiring other treatment strategies, 5 presumably the biggest challenge is to design a drug that is delivered to the mitochondria of all tissues that require enzymatic activity (including the brain) without inducing any off-target effects. While some studies might be ongoing, to the best of our knowledge, no studies have yet reported results of a clinical trial with gene therapy, mRNA supplementation or enzyme replacement therapy in PA or MMA patients, nor are there any case reports of hepatocyte transplantation in PA or MMA. Despite the fact that we assume that these reports will be published in the coming years and that the results will be positive, it will still take some time before these treatment strategies can be incorporated in standard clinical care for PA and MMA patients.

### 4.1.3 Unexplored options

In Figure 1 we demonstrate that several therapeutic targets have remained unexplored. For example, to decrease the third supply route that comprises approximately 25% of the total load of propionyl-CoA, 13,14 odd-chain fatty acid and/or cholesterol restriction can be explored. To increase disposal of toxic metabolites, we could try to identify compounds that induce glycine N-acetyltransferase activity. In addition, we could search for nontoxic scavengers of 2-methylcitric acid, methylmalonic acid, propionic acid and possibly 3-hydroxypropionic acid.

For therapeutic targets that already have been explored, there might be additional treatment options. For example, due to side effects of varying severity, treatment with metronidazole is sometimes (temporarily) discontinued in PA and MMA patients. Investigation of antibiotics with less severe side effects that effectively reduce the production of propionyl-CoA derived from anaerobic bacterial fermentation, could serve more continuous reduction of the propionyl-CoA load originating from the gut.

### 4.2 Treatment strategy 2: prevent or treat mitochondrial energetic failure and increase of ROS formation

#### 4.2.1 Current treatment strategies

There are no current treatment strategies specifically aiming to prevent or treat energetic failure and increase of ROS formation described in the guideline for PA and MMA patients 49 (Table 1).

#### 4.2.2 Experimental treatment strategies

Several experimental treatment strategies aim to prevent or treat mitochondrial failure. This aim can be attained by supplementation of anaplerotic substances, supplementation of cofactors for enzymes with decreased activities, supplementation of anti-oxidants and supplementation of electron transfer mediators. Regarding anaplerotic substances, citric acid (Figure 2, Table 1, TT#9) has been used in a clinical trial with PA and MMA patients, 85 resulting in increased citric acid intermediates and possibly also in a reduced number of hospitalizations. In addition, succinate (TT#8) has been tested for neuroprotection in methylmalonic acid-induced cognitive problems 84 and seizures 83 in rats, resulting in complete reduction of the induced phenotype. The energetic substrate creatine (TT #10) attained similar effects as succinate, possibly due to restoring intra-mitochondrial high energy phosphate levels. 84

Efficiency of cofactor supplementation for enzymes with decreased activities has also been explored. Pyridoxine
(vitamin B6) was used in rats with methylmalonic acid-induced seizures, resulting in complete reduction of the phenotype by preventing methylmalonic acid-induced inhibition of glutamic acid decarboxylase (TT#11). Thiamine (vitamin B1) is used in patients with lactic acidosis due to a (relative) thiamine deficiency, resulting in reduced lactate levels by increasing pyruvate dehydrogenase activity (TT#12).

In addition, the effects of many anti-oxidants are explored (TT#14). Ascorbic acid (vitamin C) was used in methylmalonic acid-induced glutathione reduction in rats with no effect. However, in methylmalonic acid-induced seizures, in cognitive problems induced by propionic acid and methylmalonic acid, and in a patient suffering from lactic acidosis due to glutathione deficiency, ascorbic acid did partially alleviate the phenotype. α-Tocopherol (Vitamin E) was also used in methylmalonic acid-induced glutathione reduction in rats, but also with no effect. It has been explored in methylmalonic acid-induced seizures and in methylmalonic acid-induced ROS formation and lipid peroxidation, resulting in reduction of the induced phenotype. α-Tocopherol in combination with coenzyme Q10 is used in patients with optic atrophy aiming to improve their sight with varying effects. Coenzyme Q10 has been reported to reverse cardiomopathy in one PA patient. It has also been used—with varying effects—in MMA patients with optic atrophy and in Mut−/− mice with renal failure (TT#13). Other explored anti-oxidants are CB agonist WIN and S-allylcysteine, glutathione, GM1 ganglioside, melatonin, mitoQ and resveratrol, in most cases resulting in a partial or complete reduction of the induced phenotype (TT#15).

The use of NMDA-receptor antagonists is explored specifically for the brain. Celecoxib is a known COX-2 inhibitor which acts as an indirect NMDA-receptor antagonist by preventing prostaglandin E2 production, a metabolite that induces decreased Na+/K+/ATPase activity. It has been successfully used in methylmalonic acid-induced seizures. Fish oil has also been investigated for neuroprotection in methylmalonic acid-induced seizures, in rats. Its positive effects are attributed to the interference with the convulsive activity of prostaglandin E2. Kynurenic acid, a direct NMDA-receptor antagonist is investigated for neuroprotection in propionic and methylmalonic acid-induced, and NMDA-receptor agonist co-induced ROS formation, lipid peroxidation and mitochondrial failure, leading to a partial reduction. MK-801, also a direct NMDA-receptor antagonist successfully reversed methylmalonic acid and propionic acid-induced seizures and methylmalonic acid and propionic acid-induced ROS formation, but it had no effect on methylmalonic acid-induced cognitive problems.

The fact that on one hand both anaplerotic substrates and cofactors result in reduction of methylmalonic acid and propionic acid-induced phenotypes, and on the other hand anti-oxidants attain the same effects, underlines an important aspect of PA and MMA pathophysiology: toxic metabolites induce both mitochondrial energetic failure and an increase of mitochondrial ROS formation, leading to lipid peroxidation and oxidative stress.

4.2.3 Unexplored options

In terms of treatment strategies, the three most important approaches are currently being explored: supplementation of anaplerotic substrates, supplementation of cofactors and supplementation of anti-oxidants. While the most important cofactors are already tested, the effects of some anaplerotic substances and anti-oxidant candidates are yet to be explored, such as fumarate, malate or α-ketoglutarate as anaplerotic substances and lipoic acid, retinol (vitamin A), β-carotene or uric acid (only when glomerular filtration rate is normal, Haijes et al), as anti-oxidants. However, it is not to be expected that these substances will achieve much better effects than the ones already investigated.

A final unexplored option is glutamate supplementation, which could provide α-ketoglutarate as anaplerotic substance for the citric acid cycle. Glutamine, but not glutamate, is found to be decreased in plasma of PA and MMA patients which has been attributed to shortage of α-ketoglutarate. Glutamine supplementation is controversial in critically ill patients, given the associated high rate of adverse outcomes that raised the hypothesis that low glutamine levels are an adaptive response. Therefore, glutamate supplementation, which was for example demonstrated to improve post-ischaemic heart function in rats, might be a better option to consider.

5 CONCLUSION

In this review we provide a systematic overview on current, experimental and unexplored treatment strategies in PA and MMA in order to provide insight in what we have to offer PA and MMA patients, now and in the future. We discerned two main treatment strategies: treat the cause, by reducing the amount of toxic metabolites, and treat the effects, by preventing or treating mitochondrial energetic failure and increase of ROS formation. It is encouraging that within these two treatment strategies, many therapy targets have already been identified (Figure 1, Figure 2), and many of these have been or are being explored, in various ways and to a greater or lesser extent. Unfortunately, available
evidence on the effectiveness of treatment strategies is often scarce, since none of them were tested in randomized clinical trials. This raises concerns, since the consensus on best practice treatment for PA and MMA, which includes a protein restricted diet with or without amino acid supplementation, a high caloric diet during illness, an antibiotic regimen, laxatives in case of constipation, hydroxycobalamin in case of cobalamin responsive MMA and possibly solid organ transplantation for severely affected patients, is not without controversy. In addition, treatment strategies that aim to prevent or treat mitochondrial failure and increase of ROS formation, demonstrate promising, but varying and limited effects.

To attain a curative treatment for PA and MMA patients, gene therapy, messenger RNA therapy and enzyme replacement therapy are most promising. If the accumulation of toxic metabolites is permanently reduced, the mitochondrial function should theoretically remain undisturbed. This allows for a less strict therapeutic regime and not only prevents mitochondrial-associated complications, but also complications that are due to protein restriction or the accumulation of acidic compounds. However, it is still a long way to go from bench to bedside. It is challenging to design a drug that is delivered to the mitochondria of all tissues that require enzymatic activity, including the brain, without inducing any off-target effects. Moreover, the therapeutic window of opportunity and the target tissue(s) are, to date, still not sufficiently understood.5

In summary, to further improve survival rate and, equally important, quality of life of PA and MMA patients, there is a need for systematic (re-)evaluation of each and everyone of the accepted and potential treatment strategies, ideally in (large) randomized clinical trials109 but if that is not feasible, in smaller trials85 or on an individual patient basis,1,3 so that we can better determine who will benefit when and how from which treatment strategy.

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CONFLICT OF INTEREST
All the authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS
All authors conceived the research, edited and reviewed the manuscript, and approved the final version. P.M.H., J.J.M.J., and N.M.V.-D. supervised the research. H.A.H. performed the review of literature and wrote the manuscript.

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This article does not contain any studies with human or animal subjects performed by any of the authors.

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