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Anaplerotic therapy in propionic acidemia

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ABSTRACT

Background: Propionic acidemia is a rare metabolic disorder caused by a deficiency of propionyl- CoA carboxylase, the enzyme converting propionyl-CoA to methylmalonyl-CoA that subsequently enters the citric acid cycle as succinyl-CoA. Patients with propionic acidemia cannot metabolize propionic acid, which combines with oxaloacetate to form methylcitric acid. This, with the defective supply of succinyl-CoA, may lead to a deficiency in citric acid cycle intermediates.
Purpose: The objective of this study was to determine whether supplements with glutamine (400 mg/kg per day), citrate (7.5 mEq/kg per day), or ornithine α-ketoglutarate (400 mg/kg per day) (anaplerotic agents that could fill up the citric acid cycle) would affect plasma levels of glutamine and ammonia, the urinary excretion of Krebs cycle intermediates, and the clinical outcome in 3 patients with propionic acidemia.
Methods: Each supplement was administered daily for four weeks with a two week washout period between supplements. The supplement that produced the most favorable changes was supplemented for 30 weeks following the initial study period and then for a 2 year extension.
Results: The urinary excretion of the Krebs cycle intermediates, α-ketoglutarate, succinate, and fumarate increased significantly compared to baseline during citrate supplementation, but not with the other two supplements. For this reason, citrate supplements were continued in the second part of the study. The urinary excretion of methylcitric acid and 3-hydroxypropionic acid did not change with any intervention. No significant changes in ammonia or glutamine levels were observed with any supplement. However, supplementation with any anaplerotic agents normalized the physiological buffering of ammonia by glutamate, with plasma glutamate and alanine levels significantly increasing, rather than decreasing with increasing ammonia levels. No significant side effects were observed with any therapy and safety labs (blood counts, chemistry and thyroid profile) remained unchanged. Motor and cognitive development was severely delayed before the trial and did not change significantly with therapy. Hospitalizations per year did not change during the trial period, but decreased significantly ($p < 0.05$) in the 2 years following the study (when citrate was continued) compared to the 2 years before and during the study.
Conclusions: These results indicate that citrate entered the Krebs cycle providing successful anaplerotic therapy by increasing levels of the downstream intermediates of the Krebs cycle: α-ketoglutarate, succinate and fumarate. Citrate supplements were safe and might have contributed to reduce hospitalizations in patients with propionic acidemia.

1. Introduction

Propionic acidemia is an autosomal recessive disorder caused by deficiency of propionyl CoA carboxylase (EC 6.4.1.3), the enzyme that converts propionyl CoA to methymalonyl CoA with the help of the cofactor biotin [1]. This conversion, which occurs in mitochondria, is part of the pathway for degradation of the amino acids isoleucine, methionine, threonine, and valine, odd chain fatty acids, and cholesterol [1]. Propionic acid also originates from the catabolism of the nucleotides thymine and uracil and from bacterial production of propionate from pyruvate in the gut [1]. Propionyl CoA is eventually converted into succinyl CoA and enters the citric acid (Krebs) cycle for energy production. Propionyl CoA carboxylase is composed of two distinct subunits: α and β, either of which can be defective in propionic acidemia [1]. As a result

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of defective propionyl CoA carboxylase, propionyl CoA accumulates and combines with oxaloacetate, another intermediate of the citric cycle, to form methylcitric acid, the diagnostic metabolite of propionic acidemia (Fig. 1). Most cases of propionic acidemia present.

with lethargy progressing to coma from 16 h to weeks after birth, depending on the severity of the enzyme impairment caused by the genetic lesion [1]. Patients can have severe hyperammonemia associated or not with metabolic acidosis [1,2]. Even when patients are rescued from the hyperammonemic coma, the prognosis is poor since patients can develop life-threatening complications such as pancreatitis or cardiomyopathy [2,3]. These complications, whose mechanism is unknown, cause severe morbidity and mortality even in optimally treated patients limiting the benefits of early diagnosis by newborn screening programs [2,3].

The mechanism at the basis of these long-term complications is unknown. It might be related to toxic effects of metabolites accumulating as a result of the metabolic block (including chronic hyperammonemia) and/or to decreased energy production (by inhibition of the Krebs cycle). In propionic acidemia, glutamine levels are low even in well-controlled patients and decrease (rather than increase) with hyperammonemia [4]. We have proposed that this glutamine paradox, also seen by other groups [5–7], could be due to a dysfunctional Krebs cycle, with deficiency of α-ketoglutarate [4]. In propionic acidemia, glutamate dehydrogenase, normally a cataplerotic enzyme favoring the exit of α-ketoglutarate as glutamate [4], works in reverse generating α-ketoglutarate from glutamate [4]. Low levels of glutamate favor the release of ammonia from glutamine to generate glutamate, explaining the association between high ammonia and low glutamine levels (Fig. 1).

Propionic acid is important in the anaplerosis (filling-up) of the Krebs cycle as indicated by the clinical improvement of patients with fatty acid oxidation disorders and pyruvate carboxylase deficiency treated with heptanoin, an odd-chain fatty acid that is metabolized to propionic acid and converted to succinyl-CoA [4,8–10]. Since such a mechanism is so effective in replenishing the Krebs cycle, its complete absence in patients with propionic acidemia, coupled with the sequestration of oxaloacetate by propionyl-CoA to form methylcitrate, should result in a severe deficiency of all intermediates of the citric acid cycle. In muscle, α-ketoglutarate is the intermediate with the lowest concentration (0.05 mmol/kg) after oxaloacetate (0.012 mmol/kg, [11,12]). α-Ketoglutarate concentration further declines with exercise [11] and is regenerated from glutamine and glutamate [11,12]. If glutamine/glutamate and α-ketoglutarate are too low, such as in propionic acidemia [4], the process might become ineffective in generating energy, possibly contributing to hypotonia and progressive organ failure.

Supplements can replenish substrates to the Krebs cycle. In patients with argininosuccinic aciduria, citrate, the intermediate with the highest concentration in the Krebs cycle (0.362 mmol/kg in muscle, [11,13]), can generate cytoplasmic aspartate to increase conjugation with citrulline and the urinary excretion of the water-soluble argininosuccinic acid [14,15]. This action does not require entry of citrate into mitochondria and this therapy is not routinely used given its modest efficacy and possible side effects (metabolic alkalosis, [14]). Citrate in combination with aspartate stabilized the metabolic control of

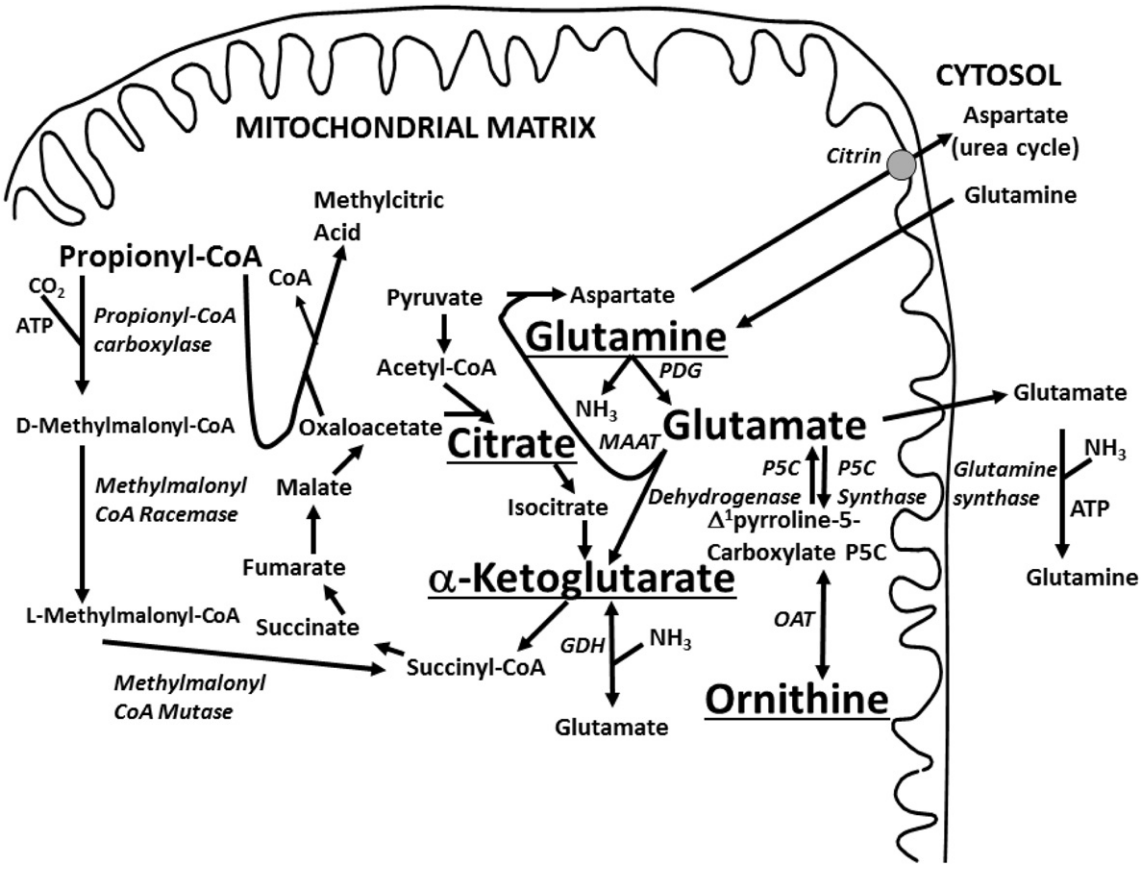

Fig. 1. Anaplerotic therapy in propionic acidemia. The supplements used in this study (ornithine alpha-ketogluratate, glutamine and citrate) are shown underlined in respect to propionic acid metabolism and the tricarboxylic acid cycle. Propionyl CoA is normally carboxylated by propionyl CoA carboxylase to become D-methylmalonyl CoA. With the action of methymalonyl CoA racemase and mutase, this produces succinyl CoA that can enter the Krebs cycle and contribute to energy metabolism. In propionic acidemia, propionyl CoA accumulates and condenses with oxaloacetate to produce methylcitric acid. The decrease in oxaloacetate and succinyl CoA can impair the Krebs cycle, reducing the concentration of alpha-ketoglutarate. This can be repleted to the expense of glutamine and glutamate. Ornithine can be converted to glutamate by the action of two enzymes, ornithine amino transferase and Δ^1pyrroline-5-carboxylate dehydrogenase. GDH: glutamate dehydrogenase; MAAT: mitochondrial aspartate amino transferase; OAT: ornithine amino transferase; PDG: phosphate-dependent glutaminase.