Pyruvate carboxylase deficiency: clinical and biochemical response to anaplerotic diet therapy

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Abstract

A six-day-old girl was referred for severe hepatic failure, dehydration, axial hypotonia, and both lactic acidosis and ketoacidosis. Biotin-unresponsive pyruvate carboxylase deficiency type B was diagnosed. Triheptanoin, an odd-carbon triglyceride, was administered as a source for acetyl-CoA and anaplerotic propionyl-CoA. Although this patient succumbed to a severe infection, during the six months interval of her anaplerotic and biochemical management, the following important observations were documented: (1) the immediate reversal (less than 48 h) of major hepatic failure with full correction of all biochemical abnormalities, (2) on citrate supplementation, the enhanced export from the liver of triheptanoin’s metabolites, namely 5 carbon ketone bodies, increasing the availability of these anaplerotic substrates for peripheral organs, (3) the demonstration of the transport of C5 ketone bodies—representing alternative energetic fuel for the brain—across the blood–brain barrier, associated to increased levels of glutamine and free γ-aminobutyric acid (f-GABA) in the cerebrospinal fluid. Considering that pyruvate carboxylase is a key enzyme for anaplerosis, besides the new perspectives brought by anaplerotic therapies in those rare pyruvate carboxylase deficiencies, this therapeutic trial also emphasizes the possible extended indications of triheptanoin in various diseases where the citric acid cycle is impaired.

Keywords: Pyruvate carboxylase deficiency; Citric acid cycle; Odd carbon fatty acids; Triheptanoin; Anaplerotic therapy; Brain metabolism

Introduction

Mitochondrial pyruvate carboxylase (PC) is most active in liver and kidney, and is a key enzyme for gluconeogenesis. It also plays an important role in anaplerosis which refills the pools of catalytic intermediates of the citric acid cycle (CAC) in other tissues such as brain, heart, kidneys, muscle, adipocytes and fibroblasts [1,2]. Additionally, it has been implicated in the proximal renal tubular reabsorption of bicarbonate [3]. PC deficiency is a very rare autosomal recessive disease, characterized by impairment of gluconeogenesis and lactate metabolism, producing severe lactic acidosis [4]. The primary result of the defect is a major deficit of oxaloacetate for the CAC [5]. This leads to profound energy deficiency due to compromised function of the CAC. Based on the severity of the clinical presentation and the biochemical disturbances, 3 phenotypes have been identified [6]. Group A or “American phenotype” is associated with psychomotor retardation and lactic acidemia...
but with a normal lactate to pyruvate ratio. Group B or “French phenotype” presents neonatally, or in early infancy, with severe metabolic ketoacidosis, lactic acidosis (elevated lactate to pyruvate ratio), hyperammonemia, hepatomegaly, and delayed myelination. These patients usually die within the first weeks of life. The third phenotype presents with metabolic acidosis but normal growth and psychomotor development. The residual enzymatic activity of PC is of no value for the distinction of these three phenotypes (always less than 5% of normal activity) [6].

Triheptanoin, a triglyceride containing three 7-carbon fatty acids (n-heptanoate), has been recently used by Roe et al. [7] in the successful treatment of long-chain fatty acid oxidation defects, that are also characterized by an underlying energy defect. Based on the very successful outcome of this study, we hypothesized that triheptanoin could be administered to patients with PC deficiency as a source of propionyl-CoA (and acetyl-CoA) inside the mitochondrion, thereby refilling the pool of catalytic intermediates of the CAC and the urea cycle while simultaneously providing fuel for gluconeogenesis.

We report the case of a newborn girl who presented with the classical clinical and biochemical findings of Group B pyruvate carboxylase deficiency “French phenotype” and who was treated with a diet containing triheptanoin.

Methods

Clinical report

E.B is the second child of healthy and non-consanguineous Caucasian parents. Pregnancy was impaired by reduced fetal active movements. Premature labor occurred at 35 weeks of gestation, requiring the administration of intravenous salbutamol and nifedipine along with preventive corticotherapy. However, after a normal full term delivery, Apgar scores were normal. Birth weight was 2700 g, length was 47.5 cm, and head circumference was 34.5 cm. Within a few hours, she developed signs of respiratory distress, dehydration (>10% of body weight), and axial hypotonia but she was cognitively interactive. At 20 h of life, severe lactic acidosis occurred. Her blood pH was 7.0, plasma lactate level was high (17 mmol/l, normal range = 0.5–2 mmol/l) associated with pronounced ketonuria but normal glyceremia while receiving standard glucose infusion. At 5 days of life, she was transferred to our hospital. Despite the high doses of intravenous sodium bicarbonate, her pH was still 7.15 with low plasma bicarbonate (11 mmol/l), high lactate (17 mmol/l) with extreme elevation of the lactate to pyruvate ratio (110, normal range = 6–14), and very low 3-hydroxybutyrate to acetoacetate ratio (0.04, normal range = 0.8–1). Plasma ammonia was 268 μmol/l (normal range = 15–45 μmol/l) with elevated alanine (958 μmol/l, normal range = 179–378 μmol/l), proline (801 μmol/l, normal = 125–279 μmol/l), citrulline (158 μmol/l, normal = 11–35 μmol/l), and lysine (713 μmol/l, normal = 121–238 μmol/l) but low glutamine (264 μmol/l, normal = 347–786 μmol/l), and glutamate (12 μmol/l, normal = 61–286 μmol/l). Urine organic acids showed elevated lactate, and acetoacetate, markedly reduced excretion of CAC intermediates, but no other abnormal organic acid excretion. She also presented with mild hepatomegaly and severe jaundice with greatly increased serum transaminases (SGOT and SGPT—40-fold and 10-fold, respectively). Clotting functions, Prothrombin Time and factor V, were <5% of normal. Cranial ultrasound revealed bilateral periventricular pseudocysts but no evidence of hemorrhage or ventricular enlargement. Cardiac examination on admission, including electrocardiogram and heart ultrasounds, was completely normal.

Dietary materials

Prior to the diagnosis, the child was treated with continuous intravenous glucose and high doses of sodium bicarbonate. Vitamin therapy (biotin, thiamine and riboflavin) was also initiated. After arrival at our unit and as soon as PC deficiency was suspected at 6 days of age, continuous drip-feeding was started with breast milk from the lactarium, with biotin (10 mg per day), thiamine (100 mg per day), and carnitine (50 mg per kg and per day). In view of the life-threatening clinical state of this patient on admission, we reviewed, with the parents, the treatment protocol and use of triheptanoin, on a compassionate basis, as part of the Food & Drug Administration IND# 59.303 (principal investigators—CRR & JMS). The parents consented to a trial of this new dietary therapy. Therefore, the triglyc eride of the 7-carbon fatty acid, n-heptanoate (provided by SASOL GmbH, Witten, Germany), was first administered on day 7 with a loading dose of 2 g/kg, followed by feeds containing 0.5 g/kg every 3 h. Due to her rapid improvement, the treatment was modified to a ketogenic diet containing about 130 calories per kg and per day with 30% represented by glucose, 30% by long-chain fatty acids, 5% by proteins, and 35% by triheptanoin—still administered every 3 h and mixed with the formula. The continuous drip-feeding was progressively stopped during the day but not during the night so that gluconeogenesis was sustained. The child was discharged from the hospital at 3 weeks of life.

Biochemical studies

Plasma lactate, pyruvate, 3-hydroxybutyrate, and acetoacetate were assayed as previously described [8]. Plasma amino acids were analysed by ion-exchange chromatography with a JEOL Aminotac Analyser [9].
Acylcarnitines were analysed by tandem mass spectrometry from Guthrie cards [10]. Urine organic acids were determined by gas chromatography–mass spectrometry after solvent extraction [11]. The concentrations of C4 and C5 ketone bodies in plasma and CSF were measured by a modification of the gas chromatography–mass spectrometry method of Leclerc et al. [12]. Pyruvate carboxylase activity was measured in cultured fibroblasts using 14C-bicarbonate as substrate [13]. Oxidation of o-deuterated fatty acids by cultured fibroblasts was evaluated using 16-[2H3]-palmitate and 7-[2H3]-heptanoate as previously described [10]. Free-GABA was measured by stable isotope dilution gas chromatography–mass spectrometry [14].

Results

Enzyme assays in cultured fibroblasts confirmed the suspected isolated pyruvate carboxylase deficiency (non detectable activity; normal range = 0.10–0.80 nmol/min/mg of protein; control = 0.30 nmol/min/mg of protein) with normal propionyl-CoA carboxylase (0.16 nmol/min/mg of protein; normal range = 0.10–0.80; control = 0.20 nmol/min/mg of protein), and normal biotinidase activity [15].

Immediate clinical and biochemical improvement with triheptanoin therapy

As early as 4 h after the loading dose of triheptanoin, there was dramatic biochemical improvement with decreased lactic acidosis and improvement of both the lactate to pyruvate ratio and the ratio of 3-hydroxybutyrate to acetoacetate due to the rapid increase of blood levels of 3-hydroxybutyrate (Fig. 1A). Marked decrease of plasma ammonia and citrulline occurred simultaneously with a corresponding increase in the level of glutamine (Fig. 1B). These findings suggested that the function of both the CAC and the urea cycle had improved. In less than 24 h, a striking decrease in serum transaminases was associated with complete normalisation of blood clotting factors. Within 2 weeks, urinary organic acid analyses confirmed the progressive decrease of lactate and ketone bodies (Table 1) along with the progressively increasing excretion of CAC intermediates (succinate, malate, fumarate) (Fig. 2A).

As a result of feeding triheptanoin, urinary excretion of derivatives of heptanoate oxidation were detected: pimelate, 6-hydroxyheptanoate, 3-hydroxypentanoate, 3-ketopentanoate, 3-hydroxypropionate, and methylcitrate (Table 1). Plasma acylcarnitine derivatives of heptanoate were also measured. There was no real increase in either pentanoylcarnitine (C5) or heptanoylcarnitine (C7) (data not shown) but propionylcarnitine (C3) increased to 4.3 μmol/l (normal upper limit 2.7 μmol/l) indicating complete beta-oxidation of heptanoate to propionyl-CoA (Table 1). These data were confirmed by in vitro analyses, including the oxidation of o-deuterated fatty acids. The patient’s fibroblasts were incubated with 16-[2H3]-palmitate illustrating the absence of any impairment of beta-oxidation. When these cells were incubated with 7-[2H3]-heptanoate, there was good production of [2H3]-propionylcarnitine without any accumulation of 7-[2H3]-heptanoyl- or 5-[2H3]-pentanoyl-carnitine (data not shown). At 120 days of age, we evaluated plasma levels of 5 carbon ketone bodies – 3-hydroxypropionate (BHP), and 3-ketopentanoate (BKP) –, and 4 carbon ketone bodies—3-hydroxybutyrate (BHB) and acetoacetate (AA) (Table 2). Substantial increases of both C5 and C4 ketone bodies were observed after meal and triheptanoin administration (data not shown), as reported with C. Roe’s patients [7] (Table 2 [7]) with BHP to BKP ratios between 1.4 and
Addition of citrate and 2-chloropropionate at 4 months of age

At 4 months of age (D120), while increasing total calories (up to 155 cal per kg and per day) with proportionally the same intake of triheptanoin (35% of total calories) but increased intake of glucose (around 40% of total calories), a progressive increase of lactate and ketone bodies was observed in the urine (Table 1) and in the plasma (Fig. 2B), associated with decreased synthesis of CAC intermediates in the urine (Fig. 2A) and low levels of C5 ketone bodies in the plasma (Table 2). Plasma bicarbonate values decreased simultaneously (Fig. 2B) and led to acute ketoacidosis at 140 days of age (D140). Severe renal tubular acidosis was detected with hyperaminoaciduria (over 15,000 μmol/mmol creatinine) and low urinary pH (5.0). Inappropriate bicarbonate excretion was observed in urine (0.3 mmol/l), associated with markedly reduced citrate excretion (0.04 mmol/day). Citrate (7.5 mmol per kg and per day) and 2-chloropropionate (50 mg per kg and per day) were therefore initiated. Both were administered simultaneously with triheptanoin therapy, along with some modifications of the diet—calories limited to 100 cal per kg, i.e., with restriction of glucose intake to 30% of total calories and normal protein intake. Most of the previous abnormal parameters corrected, as evidenced by normal plasma bicarbonate and decreased plasma lactate (Fig. 2B). Citrate supplementation was associated with a significant increase in excretion of CAC intermediates and a marked increase in plasma glutamine levels (Figs. 2A and B) which correlated with a surprising elevation of C5 ketone bodies (BHP and BKP) in the plasma (Table 2). Within a few days, renal tubular acidosis vanished with normal pH in the urine (6.5), and citrate excretion normalized (1.65 mmol/day). Excretion of 3-hydroxypropionate and methylcitrate in the urine remained stable (around 100 μmol/mmol creatinine) with the triheptanoin diet (Table 1). Despite the new and stable metabolic situ-
Table 2
Selected metabolites in plasma and CSF: triheptanoin (THN) and citrate therapy

<table>
<thead>
<tr>
<th>Days on THN</th>
<th>Plasma (mmol/l)</th>
<th>CSF (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lactate (0.6–2.2)</td>
<td>Pyruvate (0.05–0.15)</td>
</tr>
<tr>
<td>Before citrate</td>
<td>5.2</td>
<td>0.62</td>
</tr>
<tr>
<td>D120</td>
<td>6.3</td>
<td>0.34</td>
</tr>
<tr>
<td>D140</td>
<td>6.5</td>
<td>0.45</td>
</tr>
<tr>
<td>On citrate</td>
<td>5.6</td>
<td>0.17</td>
</tr>
<tr>
<td>D142</td>
<td>2.7</td>
<td>0.17</td>
</tr>
<tr>
<td>D145</td>
<td>3.4</td>
<td>0.19</td>
</tr>
</tbody>
</table>

* f-GABA = free GABA, μmol/l.

Discussion

Pyruvate carboxylase is a key enzyme in gluconeogenesis and anaplerosis that refills the pool of CAC intermediates. Its deficiency leads to a life-threatening lactic and keto-acidosis, resulting in early death in the neonatal form of the disease. The patient reported here was clinically and biochemically, typical of the severe Group B, biotin-unresponsive, PC deficiency, without any residual enzymatic activity.

The currently recommended dietary treatment for PC deficiency offers limited options since a high-fat diet would increase ketoacidosis, while a high carbohydrate diet would exacerbate lactic acidosis [16]. Oxaloacetate can be made either from aspartate through transamination, or from citrate through the action of citrate lyase. Therefore, large doses of these oxaloacetate precursors have been used in the treatment of PC deficiency by Ahmad et al. [17]. Although lactic acidosis improved and urinary metabolites of the CAC reappeared, oxaloacetate was still insufficiently available resulting in persistent excess of intramitochondrial acetyl-CoA, and so, persistent ketosis. Moreover, in other cases, neurologic outcome was usually very poor with progressive brain atrophy as shown by successive MRI. Nyhan et al. [18] recently tried orthotopic liver transplantation with significant effect on ketosis but little effect on lactic acidosis and no significant improvement of the neurologic status.

These different treatments were attempted in order to compensate for the impairments affecting the CAC, gluconeogenesis, and the urea cycle, but they have been largely unsuccessful in altering the rapid and fatal clinical course of this severe phenotype.

Very recently, Roe et al. [7] suggested that the impairment in energy observed in defects of long-chain fatty acid oxidation resulted from deficiency in the oxidation of acetyl groups in the CAC because of the impaired ability to refill the pool of catalytic intermediates of the CAC (anaplerosis). They also hypothesized that replacing dietary medium even-chain fatty acids—giving only
stable excretion of 3-hydroxypropionate and methylcitrate overload with the triheptanoin diet, as indicated by the increase in the C5 ketone bodies derived from the oxidation of heptanoate in the liver (BHP and BKP) can exert beneficial effects in various organs—including the central nervous system—by providing anaplerotic propionyl-CoA and acetyl-CoA for the CAC, after their potential transport through the blood–brain barrier. Because of the observation of the same deficit of CAC intermediates in PC deficiency, and encouraged by the very successful outcome of C. Roe’s experience with fatty acid oxidation disorders, we initiated a diet treatment containing triheptanoin to our patient.

After a very short interval (only 4 h), we observed indirect evidence of enhanced synthesis of oxaloacetate through normalization of ammonia and amino acid profiles, but also through the correction of the NADH/NAD ratio in both cytosol and mitochondrion as reflected by the decreased lactate to pyruvate ratio and the increased 3-hydroxybutyrate to acetoacetate ratio (Fig. 1A). Despite the persistence of more moderate lactate acidemia (about 4–5 mmol/l), the following weeks confirmed the restoration of the CAC function—through the maintenance of normalized parameters in the plasma and urine. These rapid biochemical corrections correlated with prompt clinical improvement due to the almost immediate disappearance of hepatic failure. The export of the 5 carbon ketones (BHP and BKP) into blood and CSF for utilization as substrates for the CAC (synthesis of acetyl-CoA and propionyl-CoA) in peripheral organs, including brain, was clearly demonstrated (Table 2). In addition, there were no signs of propionyl overload with the triheptanoin diet, as indicated by the stable excretion of 3-hydroxypropionate and methylcitrate in the urine (Table 1).

However, after 4 months, biochemical parameters indicated incomplete metabolic correction based on the progressive decrease in CAC intermediates and bicarbonate, associated with increased lactate and ketone bodies in plasma and urine (Figs. 2A, B and Table 1). Insufficient intake of triheptanoin seemed to be an unlikely explanation since the dose administered here were mildly higher than for C. Roe’s patients. Apart from the common features of PC deficiency type B, our patient also had impaired renal function—supporting the proposed role of PC in the tubular reabsorption of bicarbonate [3]. Moreover, the main hepatic failure at birth, completely reversed by the initiation of triheptanoin, could be very suggestive of an important utilization of heptanoate’s derivatives by the liver itself. In order to enhance the CAC’s output, citrate was therefore added to triheptanoin, along with continuous enteral feeding—with lower calories and glucose. To decrease lactate production, 2-chloropropionate [19]—a non-physiological activator of both PDH and α-ketoglutarate dehydrogenase—was also introduced. This combination produced prompt recovery of the patient within a few days, together with the resolution of lactic- and keto-acidosis as well as renal tubular acidosis. (Fig. 2B) Simultaneously, increased excretion of CAC intermediates in the urine were observed along with increased levels of plasma glutamine (Figs. 2A and B) that was also increased in the CSF. Moreover, the elevation of the C5 ketone bodies in the plasma—with the same proportional intake of triheptanoin—as well as their striking elevation in the CSF, suggested an increased availability of triheptanoin’s metabolites for peripheral organs on citrate therapy (Table 2). Nevertheless, at 6 months of age, the child developed an infection, as observed at autopsy, that prevented, for the first time, appropriate intestinal absorption. It resulted in a devastating ketoacidotic episode, unresponsive to therapy, so that the patient died within a few days. Despite the good metabolic balance obtained with the administration of triheptanoin, citrate, and 2-chloropropionate, this fatal event emphasized the difficulty to maintain a stable metabolic condition in case of acute intercurrent events, mainly as intravenous emulsion was not available.

The impaired synthesis of glutamine induced by PC deficiency deprives neurons of this critical amino acid that may result in neuronal death secondary to decreased neurotransmitter pools of glutamate and GABA [20–22]. Additionally, the impairment of anaplerosis in the CNS may be related to inadequate myelin formation. These findings are associated with the severe psychomotor delay and histological data present in untreated PC deficiency. Together with the alterations in the migration of cells and the formation of myelin usually observed [23], important degenerative lesions such as cystic periventricular leukomalacia are also observed, suggesting events that can occur early in pregnancy, in these patients [24].

Our patient had reduced fetal movements, and bilateral periventricular pseudocysts at birth, suggesting a role for pyruvate carboxylase in brain development. Since fatty acids cannot be oxidized in the CNS, fuel is restricted to mainly glucose, ketone bodies (OHB and AcAc) [25–27] and amino acids. Hepatic oxidation of heptanoate led to the production of BHP and BKP that crossed the blood–brain barrier [28] providing substrate for the CNS (Table 2). At 3 months of age, despite persistence of mild axial hypotonia, psychomotor development, EEG, and MRI were age-appropriate, mainly with no apparent derangement of myelination. At 6 months of age, autopsy data confirmed the absence of any degen-
CoA eliminated the deenhanced production of succinyl-CoA from propionyl-CoA as substrates for the CAC. The presumed (inside the mitochondrion) to acetyl-CoA and propionyl-CoA for cerebral anaplerotic therapy” involving oxidation of heptanoate infant provides data that support the concept of “anaplerotic lesion and revealed diffuse age-appropriate myelination in contrast to all previously reported patients. Despite various therapeutic attempts [29], CSF levels of glutamine and GABA also remained low in other patients with this disorder. The addition of citrate with triheptanoin in our patient led to a significant increase of glutamine and free GABA together with the 10-fold increase of the C5 ketone bodies in the CSF (Table 2). The availability of BHP and BKP for cerebral anaplerosis was associated with a surprising absence of brain pathology in this patient.

In conclusion, the prompt and stable recovery of this infant provides data that support the concept of “anaplerotic therapy” involving oxidation of heptanoate (inside the mitochondrion) to acetyl-CoA and propionyl-CoA as substrates for the CAC. The presumed enhanced production of succinyl-CoA from propionyl-CoA eliminated the deficit of oxaloacetate permitting normal operations for the CAC, the dicarboxylate (malate) shuttle, the urea cycle, and gluconeogenesis. Through the hepatic synthesis of C5 ketone bodies and their transport to the CNS, triheptanoin appears to provide important support for brain metabolism in PC deficiency as an alternate energy fuel when glucose metabolism is severely impaired. Therefore, the demonstrated capacity of fully reversing the energy disturbances when pyruvate carboxylase is deficient, offers a potentially useful treatment alternative for various—inherited or acquired diseases—in which the CAC is impaired, including those affecting the brain.

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