A patient with severe pyruvate carboxylase deficiency presented at age 11 weeks with metabolic decompensation after routine immunization. She was comatose, had severe lactic acidemia (22 mM) and ketosis, low aspartate and glutamate, elevated citrulline and proline, and mild hyperammonemia. Head magnetic resonance imaging showed subdural hematomas and mild generalized brain atrophy. Biotin-unresponsive pyruvate carboxylase deficiency was diagnosed. To provide oxaloacetate, she was treated with high-dose citrate (7.5 mol/kg−1/day−1), aspartate (10 mmol/kg−1/day−1), and continuous drip feeding. Lactate and ketones diminished dramatically, and plasma amino acids normalized, except for arginine, which required supplementation. In the cerebrospinal fluid (CSF), glutamine remained low and lysine elevated, showing the treatment had not normalized brain chemistry. Metabolic decompensations, triggered by infections or fasting, diminished after the first year. They were characterized by severe lactic and ketoacidosis, hypernatremia, and a tendency to hypoglycemia. At age 3½ years she has profound mental retardation, spasticity, and grand mal and myoclonic seizures only partially controlled by anticonvulsants. The new treatment regimen has helped maintain metabolic control, but the neurological outcome is still poor. Am. J. Med. Genet. 87:331–338, 1999. © 1999 Wiley-Liss, Inc.

KEY WORDS: pyruvate carboxylase; anaplerotic role; treatment; citrate; aspartate; neurological outcome

INTRODUCTION

Pyruvate carboxylase (E.C. 6.4.1.1) is a mitochondrial homotetrameric matrix enzyme that catalyzes the ATP-dependent fixation of CO2 to pyruvate yielding oxaloacetate. This reaction is important for gluconeogenesis and lipogenesis and is essential to the function of the Krebs cycle (Fig. 1). The pyruvate carboxylase gene, located on chromosome band 11q13.4-q13.5, codes for a cDNA of 3,537 nucleotides, which encodes a 1,178 amino acids protein [Wexler et al., 1994]. The single 130-kDa subunit contains covalently attached biotin and all catalytic and regulatory functions.

Deficiency of pyruvate carboxylase is an autosomal recessive disease with a very low incidence in most populations (1:250,000 live births) [Robinson, 1995]. This deficiency results in impairment of gluconeogenesis and lactate metabolism and also compromises the anaplerotic role in providing oxaloacetate to the Krebs cycle. Based on the severity of the clinical presentation and the biochemical disturbances, two clinical forms have been described representing the extremes of a spectrum of clinical variation [Robinson, 1995]. The milder form A presents in infancy with delayed neurological development, chronic lactic acidemia, and a normal lactate to pyruvate ratio. Plasma levels of alanine and proline are elevated whereas citrulline and lysine levels are normal. Longer survival but with severe clinical sequelae is common in the mild form A of pyruvate carboxylase deficiency. The complex form B presents neonatally or in early infancy with severe metabolic acidosis, lactic acidosis, ketosis, and hepatomegaly. The lactate to pyruvate ratio is elevated, and the β-hydroxybutyrate to acetoacetate ratio is reduced. Glutamine and aspartate levels are reduced, and the insufficient aspartate results in elevated levels of citrulline and lysine. The impairment of the urea cycle...
results in hyperammonemia. Most patients die neonatally, and the disorder is usually fatal by age 6 months [Robinson, 1995; Pineda et al., 1995]. The difference between these two types is related to the degree of residual enzyme activity. At least half the patients of the severe type B have no cross-reactive protein, whereas cross-reactive protein is always present in patients with the milder type A [Robinson et al., 1987].

We describe a patient, now age 3½ years, with biochemical findings of the complex type B deficiency of pyruvate carboxylase. She has remained metabolically stable with aggressive treatment with high doses of aspartate and citrate to support the deficient anaplerotic role and continuous glucose supplementation to avoid gluconeogenic stimuli. Nevertheless she has profound mental impairment.

**CLINICAL REPORT**

The patient is the first child of healthy nonconsanguineous Caucasian parents with an unremarkable family history. She was the product of a normal pregnancy and delivery born at term with normal Apgar scores. After an uneventful early infancy, she developed a fever after routine immunizations at age 11 weeks. Within 24 hours she was lethargic and limp with labored breathing and at presentation was comatose. Her pH was 6.9, and the plasma lactate level peaked at 22 mM (normal range: 0.5–3.1 mM). Initially the lactate to pyruvate ratio was 27, the next day it was 54 (normal: < 25) (Table I). There was pronounced ketonuria. Ammonia was 63 μM (normal range: 11–36 μM), glucose was normal at 7.2 mM (130 mg/dl). She had myoclonic movements, but electroencephalogram showed no electrographic seizures. Cranial magnetic resonance imaging showed an old right frontal subdural hematoma and a recent right occipital parietal subdural hematoma. There was diffuse cerebral atrophy, but there were no lesions in the brainstem or basal ganglia (Fig. 2 A,B). Results of blood clotting studies (PTT, APTT, and fibrinogen) and liver enzyme levels (transaminases, bilirubin, and alkaline phosphatase) were normal.

Plasma amino acids on admission showed elevated citrulline, lysine, and alanine (Table I). Urine organic acids were measured by gas chromatography mass spectrometry as their ethoxyamine- and trimethylsilyl-
Pyruvate Carboxylase Deficiency

TABLE I. Plasma and CSF Metabolite Levels*

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Day 1 Plasma</th>
<th>Day 2 Plasma</th>
<th>Day 6 Plasma</th>
<th>Day 14 Plasma</th>
<th>Day 14 CSF</th>
<th>Follow-up at age 23 month Plasma</th>
<th>Follow-up at age 23 month CSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate</td>
<td>11.1–22.5</td>
<td>12.1</td>
<td>8.7</td>
<td>8.0</td>
<td>6.6</td>
<td>5.7</td>
<td>6.9</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>0.41</td>
<td>0.23</td>
<td>0.30</td>
<td>0.34</td>
<td>0.41</td>
<td>0.41</td>
<td>0.28</td>
</tr>
<tr>
<td>L/P</td>
<td>27</td>
<td>52</td>
<td>26</td>
<td>19</td>
<td>13.9</td>
<td>24.6</td>
<td>&lt; 25</td>
</tr>
<tr>
<td>Alanine</td>
<td>766.6</td>
<td>283.7</td>
<td>741</td>
<td>128.6</td>
<td>1193</td>
<td>102.8</td>
<td>143–493</td>
</tr>
<tr>
<td>Proline</td>
<td>597.7</td>
<td>133.5</td>
<td>308</td>
<td>0</td>
<td>366.7</td>
<td>0</td>
<td>52–298</td>
</tr>
<tr>
<td>Lysine</td>
<td>743.5</td>
<td>175.3</td>
<td>201</td>
<td>46.6</td>
<td>174.4</td>
<td>35.7</td>
<td>52–196</td>
</tr>
<tr>
<td>Citrulline</td>
<td>114.8</td>
<td>6.6</td>
<td>19.6</td>
<td>2.8</td>
<td>13.3</td>
<td>0</td>
<td>3–35</td>
</tr>
<tr>
<td>Aspartate</td>
<td>5.4</td>
<td>15</td>
<td>22</td>
<td>1.5</td>
<td>44.4</td>
<td>1.9</td>
<td>0–23</td>
</tr>
<tr>
<td>Glutamine</td>
<td>572.9</td>
<td>212</td>
<td>273.2</td>
<td>259.4</td>
<td>642.6</td>
<td>346.8</td>
<td>248–1182</td>
</tr>
</tbody>
</table>

* L/P, lactate to pyruvate ratio.

esters (Fig. 3A). They showed elevated lactate, pyruvate, 3-hydroxybutyrate, acetocacetate, and 2-hydroxybutyrate. The level of 2-ketoglutarate was low; and no other Krebs cycle metabolites were present. There was no methylcitrate or 3-hydroxypropionate, and 3-hydroxyisovalerate was normal. Plasma acylcarnitine profile showed increased acetylcarnitine but was otherwise normal. Fibroblast enzyme assays confirmed the suspected isolated pyruvate carboxylase deficiency: pyruvate carboxylase: 4 pmol/min−1/mg−1 protein (control range 128–573); propionyl-CoA carboxylase 320 pmol/min−1/mg−1 protein (control range 71–250) (T.P. Le, University of California at San Diego, San Diego, CA). Biochemical effect was observed. Biotin was maintained at 30 mg/day until 2 years 2 months because of persistently low plasma levels. Continuous gastrostomy feedings provide 45 kcal/kg−1/day−1 and 100 cc/kg−1/day−1 fluid.

**Treatment Results**

After gaining control of the acute metabolic decompensation, metabolic stability was maintained. When well and clinically stable, plasma lactate levels ranged between 3.8 and 8 mM, and she had mild to moderate ketonuria. During the first year she required four hospitalizations for metabolic decompensation triggered by intercurrent infections or by fasting. During these episodes there was severe lactic acidosis, ketoacidosis, a tendency towards hypoglycemia, and hypernatremia because of reduced oral fluid intake. These metabolic decompensations were treated with continuous intravenous glucose (6–9 mg/kg−1/min−1) and intravenous citrate and aspartate, the doses of which were limited by the amount of sodium. Whereas normal levels of plasma aspartate were easily maintained, high doses were required to normalize urine aspartate excretion (10 mmol/kg−1/day−1). With this dose, plasma amino acid levels normalized. However, CSF glutamine level remained low, and lysine level elevated (Table I). On this high-dose regimen of citrate (7.5 mmol/kg−1/day−1) and aspartate (10 mmol/kg−1/day−1), she had no primary metabolic decompensations over the second year. Occasional hospitalizations have been secondary to respiratory infections subsequently leading to metabolic decompensation.

At age 2 years, she was hospitalized twice for severe hypokalemia and hypomagnesemia associated with prolonged functional ileus. In her third year, renal disease with hematuria and renal tubular dysfunction with electrolyte loss and generalized amino aciduria developed. Serum levels of urea and creatinine remain normal.

At age 8 months her cholesterol level was discovered to be low at 65 mg/dl−1. It was 58 mg/dl−1 at age 15 months and 77 mg/dl−1 at age 19 months. Cholesterol intake was very low. The 7-dehydrocholesterol level was normal at 0.10 μg/ml−1 (normal 0.10 ± 0.05) (R. Kelley, Kennedy Krieger Institute, Baltimore). Urinary...
Fig. 2. T1 weighted images of the head at presentation at age 11 weeks (transverse view A and sagittal view B) show an old chronic right frontal subdural hematoma and a smaller right posterior parietal-occipital subdural hematoma. There is cortical atrophy, particularly in the frontotemporal region, and a thin corpus callosum, but no lesion in the basal ganglia or brain stem. At age 22 months (transverse view C and sagittal view D), elliptical head shape is seen because of lambdoidal craniosynostosis. There is cortical atrophy and delayed myelination, but normal basal ganglia, brain stem, and cerebellum.
Fig. 3. Urine organic acids analysis before treatment (A) shows absence of Krebs cycle metabolites in the presence of massive lactate, pyruvate, acetoacetate, and β-hydroxybutyrate. After treatment (B), Krebs cycle metabolites 2-ketoglutarate, citrate, fumarate, and succinate are present in addition to large amounts of lactate, pyruvate, and ketones. Lactate (1), pyruvate (2), 2-hydroxybutyrate (3), 3-hydroxybutyrate (4), acetoacetate (5), urea (6), succinate (7), fumarate (8), adipic (9), 2-ketoglutarate (10), 4-hydroxyphenylacetate (11), cis-aconitate (12), hippurate (13), citrate (14), 4-hydroxyphenyllactate (15), 4-hydroxyhippurate (16), and tetracosane (17) (internal standard at 30 mg/mmol creatinine).
mevalonate concentration, measured using a radioenzymatic double isotope dilution method [Pappu et al., 1989], was 101 nmoles/kg⁻¹/day⁻¹, which is significantly higher than that observed in normal infants 47 ± 24 nmoles/kg⁻¹/day⁻¹ (mean ± SEM) (Pappu AS, Connor WE, unpublished observations) and normal adults 23 ± 2 nmoles/kg⁻¹/day⁻¹ (mean ± SEM). Parenteral cholesterols were 164 mg/dl⁻¹ and 135 mg/dl⁻¹, and LDL-cholesterol levels were 102 mg/dl⁻¹ and 63 mg/dl⁻¹ for the father and the mother, respectively. Cholesterol supplementation was begun at age 28 months at a dose of 50 mg/kg⁻¹/day⁻¹ and slowly increased to 150 mg/kg⁻¹/day⁻¹ in an attempt to normalize plasma cholesterol levels. Plasma cholesterol levels ranged between 67 and 108 mg/dl⁻¹. The parents noted subjective improvement in activity level and alertness, but no objective improvement in neurological development was seen.

**Long-Term Outcome**

Neurological outcome has remained poor despite the relative metabolic stability. The patient has profound mental retardation with a developmental age of 3 months at a chronological age of 3½ years. She has spasticity and severe axial hypotonia. She developed myoclonic seizures at age 7 months, which are partially controlled with clonazepam and lamotrigine. She continues to make very slow progress with incomplete head control and increased responsiveness to her environment. Follow-up brain magnetic resonance imaging examination at age 22 months showed progressive cerebrbral atrophy (Fig. 2 C,D). Myelination was delayed, although it had advanced with age. There were no brain stem or basal ganglia lesions. Premature lambdoid craniosynostosis developed during the first year of life. Hypoventilation of both a central and peripheral component, secondary to significant hypotonia, necessitated nighttime assisted positive airway pressure support. Feeding is by continuous gastrostomy because of the severe neurological problems. She continues to gain weight at 45 kcal/kg⁻¹/day⁻¹, and she is on the 95th centile for weight and between the 50th and 75th centiles for length.

To examine possible mechanisms for the poor neurological outcome, CSF was further analyzed at age 23 months (Table I). Because aspartate is also necessary for purine synthesis, CSF was analyzed for 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) and 4-N-succinyl-5-aminoimidazole-4-carboxamide ribonucleotide (SAICAR) levels. Normal levels were found (G. Van den Berghe, Institute of Cellular Pathology, UCL, Louvain-la-Neuve, Belgium).

**DISCUSSION**

Most patients with pyruvate carboxylase deficiency type B die within the first 3 months of life from overwhelming metabolic lactic acidosis and ketoacidosis and dysfunction of the urea cycle [Robinson, 1995]. Longer survival has been reported for patients with the milder, type A, pyruvate carboxylase deficiency. The patient reported here is biochemically similar to type B pyruvate carboxylase patients with elevated citrulline, lysine, and proline, and elevated lactate to pyruvate ratio. She is somewhat milder than most type-B patients. At initial presentation our patient had two subdural hematomas of differing age, leading to the initial suspicion of child abuse, which was subsequently ruled out. A subarachnoid hemorrhage was suspected in one previously reported patient with pyruvate carboxylase deficiency [Rutledge et al., 1989]. Pyruvate carboxylase joins glutaric aciduria in the list of inborn errors of metabolism that can present with subdural hematomas and effusion [Hoffmann et al., 1996]. Coagulopathy [Saudubray et al., 1976; Wong et al., 1986] and liver dysfunction [Haworth et al., 1981; Greter et al., 1985; Wong et al., 1986], previously reported in pyruvate carboxylase deficiency, can potentially contribute to this complication but were not evident in our patient. Craniosynostosis, which has not been reported previously, is most likely secondary to the cerebral atrophy.

The treatment of pyruvate carboxylase deficiency has included the provision of pharmacological doses of co-factors involved in pyruvate metabolism and the substitution of missing end-products. Treatment with high doses of biotin has been very successful in some cases of partial pyruvate carboxylase deficiency [Higgins et al., 1994], even without a mutation in the biotin binding region [Higgins et al., 1997], and should be tried first. Vitamins, which are cofactors in other enzymes of pyruvate metabolism such as thiamin and lipoic acid, have generally been ineffective. They could have a place in the general promotion of anabolism. The avoidance of fasting episodes with its attendant gluconeogenic stimulus is essential. Our patient initially showed a tendency to hypoglycemia requiring provision of additional carbohydrate. Almost all metabolic decompensations were induced by prolonged fasting or infections, situations in which gluconeogenesis is induced. Yet treatment with continuous glucose alone has generally been insufficient to control metabolic decompensations particularly in type-B patients. Dietary treatment provides limited options because a fat-enriched diet will increase the ketoacidosis and high carbohydrates increase the lactic acidosis.

Oxaloacetate is the primary missing end product, and a deficiency of its anaplerotic role in the formation of citrate in the Krebs cycle and the formation of argininosuccinic acid in the urea cycle is considered central to the biochemical disturbances described in the complex form, type B, of pyruvate carboxylase deficiency [De Vivo et al., 1977]. In the cytosol, oxaloacetate can be made from asparagine through transamination and from citrate through the action of citrate lyase (Fig. 1). Various doses and combinations of aspartate, asparagine, glutamate, and glutamine have been used to provide oxaloacetate in pyruvate carboxylase deficiency [Baal et al., 1981; Oizumi et al., 1983, 1984]. Aspartate was considered the most effective component. This treatment has led to stabilization of the metabolic acidosis [Baal et al., 1981; Oizumi et al., 1983] and of progressive neurological deterioration [Baal et al., 1981]. Cessation of therapy resulted in a considerable increase in lactic acidosis [Baal et al., 1981]. Citrate therapy at 1.7 mEq/kg⁻¹/day⁻¹ also re-
resulted in reduction of lactic acidosis [Wong et al., 1986]. In our patient, citrate therapy alone initially improved the lactic acidosis and showed return of Krebs cycle metabolites in the urine. However on day 6 the inappropriately low alanine level in the presence of markedly elevated lactate (Table I) indicated that the aspartate availability for transamination was markedly insufficient, and aspartate treatment was added. In order to maintain metabolic stability and normalize aspartate levels, the dose of both citrate and aspartate was gradually increased to more than 20 times higher than previously reported. This aggressive treatment has stabilized the metabolic decompensations and improved survival.

Aspartate and citrate were given as sodium salts. The large amount of sodium limited the dose that could be used, and hypernatremia developed repeatedly whenever fluid intake decreased. After 2 years, she developed episodes of ileus with hypomagnesemia and hypocalcemia and also a renal disease with hematuria and tubular dysfunction. A renal tubular defect with hypernatremia, renal tubular acidosis, and cyturiuria has been reported in one patient with pyruvate carboxylase deficiency treated with aspartate [Oizumi et al., 1983]. It is not known whether the renal disturbances in our patient reflect a nephrotoxic effect of this treatment, or a new renal complication of the pyruvate carboxylase deficiency, possibly similar to the previously reported hepatic dysfunction [Haworth et al., 1981; Greter et al., 1985; Wong et al., 1986].

With these large doses of oxaloacetate precursors, plasma aspartate levels normalized, and there was good excretion of the Krebs cycle metabolites fumarate, succinate, and 2-ketoglutarate. Nevertheless, ketosis persisted, indicating that oxaloacetate was still insufficiently available inside the mitochondria. Acetyl-CoA that finds insufficient oxaloacetate for citrate formation and catabolism in the Krebs cycle, is used for ketone body formation (Fig. 3). Mitochondrial acetyl-CoA is transported from the mitochondria to the cytoplasm as citrate where it is the substrate for carboxylation to malonyl-CoA, which down-regulates carnitine palmitoyltransferase I, the primary regulator in hepatic fatty acid oxidation and ketogenesis. Insufficient mitochondrial oxaloacetate for citrate synthesis and acetyl-CoA transport results in excess intramitochondrial acetyl-CoA leading to persistent excessive ketosis [De Vivo et al., 1977]. In our patient, despite treatment, significant ketosis persisted.

Furthermore, aspartate is required in citrulline synthesis and in sarcosine synthesis from lysine. Despite normalization of plasma citrulline levels, arginine remained low, and supplementary treatment was necessary. Arginine used alone had little effect in previous patients [Pollock et al., 1986; Haworth et al., 1991]. Levels of CSF lysine and glutamine remained abnormal despite normal plasma levels. These data illustrate the difficulties in transfer of the oxaloacetate precursors provided in this treatment across the mitochondrial membrane and across the blood-brain barrier.

This cytosolic acetyl-CoA is also the substrate for cholesterol synthesis, and insufficient cytosolic acetyl-CoA could result in ineffective cholesterol synthesis. Indeed, cholesterol levels were consistently low in our patient. Since several cholesterol synthesis deficiency syndromes have neurological symptoms, this finding was further investigated. Changes in the concentration in plasma and urine of mevalonic acid, a product of 3-hydroxy-3-methylglutaryl-coenzyme A reductase and a committed precursor of cholesterol biosynthesis, reflect parallel changes in the rates of hepatic and whole body cholesterol biosynthesis [Pappu et al., 1989]. Hence 24-hr excretion of urinary mevalonate is used as an indicator of the rate of whole body cholesterol biosynthesis. Mevalonate excretion was elevated in our patient, indicating that the rate of cholesterol biosynthesis appears to be higher in this patient. High rates of cholesterol biosynthesis and low plasma cholesterol levels are also observed in patients with abetalipoproteinemia, a genetic disorder characterized by complete absence of apoB containing lipoproteins [Illingworth et al., 1989]. Here, the low LDL-cholesterol level in the mother strongly suggests that the cause of the hypocholesterolemia is the result of coincidental hypobetalipoproteinemia. The elevated mevalonic acid excretion illustrates that the patient was able to compensate with increased endogenous cholesterol synthesis. This indicates that either the pool of acetyl-CoA was still sufficient for the cholesterol synthesis or a compensatory increase in the precursor pool by other metabolic pathways. High levels of cholesterol in a previously reported patient [De Vivo et al., 1977] further support the adequate capacity of cholesterol synthesis in this disorder. As expected, exogenous cholesterol supplementation had no objective effect on clinical neurological symptoms.

Despite this intensive treatment program, neurological outcome has remained very poor. Successive MRI evaluations of the brain have documented progressive brain atrophy. Myelination, although advancing with age, remains less than normal. Neuronal energy deficit has generally been considered the cause of the brain degeneration. Surprisingly, no changes were seen in the basal ganglia and brainstem, regions frequently involved in disorders of energy production accompanied by lactic acidosis. Other possible contributing causes for the poor neurological outcome include disturbances in brain amino acids, in particular deficiency in the neurotransmitters glutamate and aspartate, and the derived neurometabolites N-acetyl-aspartate, N-acetylaspartyglutamate, and glutamine. In CSF, aspartate levels were normal, but glutamine was low. Low glutamine levels have previously been reported in the brain [Perry et al., 1985]. Pyruvate carboxylase and glutamine synthetase (E.C. 6.3.1.2) are both located primarily in the astrocytes, and pyruvate carboxylase contributes to the synthesis of both glutamate and glutamine [Gamberino et al., 1997]. Glutamine synthesis through glutamine synthetase requires ATP in addition to ammonia and glutamate. Glutamate levels and ammonia were normal in the brain [Perry et al., 1985], suggesting insufficient ATP in astrocytes for glutamine synthesis. Aspartate is also important in purine synthesis, disorders of which result in severe neurological problems. Analysis of CSF purines during treatment
did not demonstrate abnormalities. In the future glutamine treatment is necessary, since glutamine deficiency has not yet been corrected in our patient.

This case illustrates that despite the efficacy of this new regimen in maintaining metabolic control in this severe condition, the neurological outcome remains very poor. It underscores the central role in pathophysiology of the dysfunction in the anaplerotic role of this enzyme in mitochondrial biochemistry, and illustrates the importance of compartmentalization, such as imposed by the blood brain barrier, in its treatment.

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REFERENCES


