The Role of Methionine in Ethylmalonic Encephalopathy with Petechiae

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Background: Among patients with ethylmalonic aciduria, a subgroup with encephalopathy, petechial skin lesions, and often death in infancy is distinct from those with short-chain acyl-coenzyme A dehydrogenase deficiency or multiple acyl-coenzyme A dehydrogenase deficiency. The nature of the molecular defect in this subgroup is unknown, and the source of the ethylmalonic acid has been unclear.

Objective: To determine whether the administration of candidate amino acids increased the excretion of ethylmalonic acid.

Design: Examination of patterns of organic acids excreted in the urine before and following loading doses of isoleucine and methionine.

Setting: General clinical research center.

Patient: An infant with ethylmalonic aciduria, global developmental delay, acrocyanosis, and intermittent showers of petechiae.

Main Outcome Measure: Excretion of ethylmalonic acid in the urine.

Results: Loading with methionine increased the excretion of ethylmalonic acid, whereas loading with isoleucine did not. Restriction of the dietary intake of methionine decreased ethylmalonic acid excretion.

Conclusion: In ethylmalonic acid encephalopathy with petechiae, methionine is a precursor of ethylmalonic acid.

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ETHYLMALONIC ENCEPHALOPATHY with petechiae is a progressive degenerative disease in which acrocyanosis and petechial skin lesions are prominent. Acute episodic metabolic crises occur in which there is acidosis and mild hypoglycemia. Neuroimaging results have revealed extensive frontotemporal atrophy and areas of high T2-weighted intensity in the basal ganglia. The defining metabolic abnormality is the excretion of large amounts of ethylmalonic acid in the urine. Excretion of adipic acid is not increased in contrast to multiple acyl-coenzyme A dehydrogenase deficiency, which has been referred to as ethylmalonic-adipic aciduria. Ethylmalonic aciduria is also encountered in short-chain acyl-coenzyme A dehydrogenase deficiency in which an initial feature is hypoketotic hypoglycemia, which is characteristic of disorders of fatty acid oxidation.

The vascular manifestations of this disorder are unusual and characteristic features. Acrocyanosis appears to be its most manifestation. The development of showers of petechiae in response to intercurrent illness has led to investigation and treatment for presumptive meningococemia. Ecchymoses and hemorrhagic streaks have also been seen, as has an infarct of the basal ganglia. Further evidence of bleeding abnormality are microscopic hematuria and hemoperitoneum. Biopsy of the skin lesions has shown only hemorrhage. Dilated tortuous retinal vessels are seen.

Cerebral abnormality is manifest early in hypotonia and delayed development and later in spasticity. Generalized seizures are associated with abnormal focal discharges on the electroencephalogram. Neurologic deterioration accelerates following intercurrent infectious illness, and most patients have died in the early years of life. It is the purpose of this report to describe a patient in whom methionine loading led to a marked increase in the excretion of ethylmalonic acid and restriction of the intake of methionine led to decreased excretion.
CASE 1

This patient was 8 months of age when she was admitted to the University of California San Diego General Clinical Research Center (Figure 1) in San Diego. She had appeared to develop normally for 3 months after which she lost the ability to control her head, reach for objects, and sit with support. By 8 months of age, she was limp, disinterested in her surroundings, and spent most of her time sleeping. Infantile spasms began 1 month prior to hospital admission. She also had absence seizures in which the eyes rolled. Striking easy bruising began at 4 months of age. Hematological investigation at a university hospital revealed a normal platelet count, prothrombin time, partial thromboplastin time, factor VIII activity, ristocetin cofactor activity, and von Willebrand factor antigen. Lactate concentrations in serum taken on 2 occasions were 45.9 mg/dL (5.1 mmol/L) and 36.9 mg/dL (4.1 mmol/L). Her pyruvate level was 0.001 mg/dL (0.17 mmol/L). Her creatine kinase level was 2100 U/L. The electroencephalographic pattern was that of hypsarrythmia. There were several generalized epileptiform abnormalities. Magnetic resonance images revealed patchy T2-weighted hyperintensities in the putamen and cortical atrophy. Muscle biopsy findings showed increased lipid droplets but no ragged red fibers; electromicroscopy results showed there were mildly increased numbers of pleomorphic subsarcolemmal mitochondria. No deletions or point mutations were found in the mitochondrial DNA of the muscle. Enzyme analysis of frozen muscle at the Molecular Cardiology Institute, Highland Park, NJ, revealed reduced activity of complex III, IV, and V. Activity of citrate synthase was not increased.

The parents were cousins in whom the mother’s paternal grandmother was the sister of the father’s maternal grandfather (Figure 2). The patient had sisters 6 and 9 years of age who were well. A 13-year-old brother was well except for myopia. A brother (case 2) died at 8 months of age of what appeared to be the same disease as in case 1.

On examination, her weight was 7.1 g (10th percentile); length, 165 cm (5th percentile); and head circumference, 42.5 cm (25th percentile). She was very floppy and unresponsive and had virtually constant infantile spasms. She had epicanthal folds, upslanting palpebral fissures, and an upturned nose with a depressed nasal bridge. Her parents noted that her facial features were similar to those of her brother (case 2) and dissimilar to her other siblings. Deep tendon reflexes were markedly increased, and there was sustained ankle clonus at the slightest touch. There was a soft systolic precordial murmur. Studies of nerve conduction velocity revealed evidence of sensory neuropathy in the legs. There were multiple cutaneous petechiae.

Her hemoglobin level was 8.5 g/L, and her hematocrit level was 27.9%. Her serum lactate concentration was 33.4 mg/dL (5.7 mmol/L). Her bicarbonate level was 15 mEq/L. Her values for sodium, potassium, and chloride were 143 mEq/L, 4.0 mEq/L, and 112 mEq/L, respectively. Her uric acid level was 0.005 mg/dL (0.27 mmol/L). The patient had a normal prothrombin time (10.9 seconds; control subject, 10.3 seconds), partial thromboplastin time (24.2 seconds; control subject, 27 seconds), bleeding time (6 minutes, 30 seconds), and von Willebrand factor antigen. Platelet counts were normal (564 × 10^3/µL). The level of plasminogen activator inhibitor –1 was markedly elevated at 35 ng/mL (reference range, 3 - 5 ng/mL).

Concentrations of amino acids in the blood and cerebrospinal fluid were normal. Examination results of the mitochondrial DNA of the serum were negative for point mutations and deletions. The plasma concentration of free carnitine was low at 14 µmol/L (reference range, 21 - 33 µmol/L). Her urinary free carnitine level was 11 mmol/mol of creatine, whereas her esterified carnitine level was 57 mmol/mol of creatine, yielding an elevated esterified carnitine–urinary free carnitine ratio of 5.2 (reference range, 0 - 3). Analysis of the organic acids of the urine revealed ethylmalonic aciduria. The level was 276 mmol/mol of creatinine (reference range, 0 - 11). The values for adipic and glutaric acids were 79 mmol/mol of creatine and 11 mmol/mol of creatine, respectively (reference ranges, 0 - 12 mmol/mol of creatine and 0 - 5 mmol/mol of creatine, respectively). An acylcarnitine profile revealed increased C4 and C5 acylcarnitine esters, as well as increased C2.
CASE 2

The infant in this case developed irritability at 2 months of age and hypotonia and myoclonic jerks a month later. He also had episodic petechiae, metabolic acidosis, and lactic acidemia. Magnetic resonance images revealed symmetric T2-weighted hyperintensities in the basal ganglia. Progressive neurodegeneration was followed by death at 8 months of age. At autopsy, the brain weighed 630 g. One half of the brain was frozen, and the other half was fixed in formaldehyde. On gross examination, the coronal sections of the fixed half of the brain appeared normal except for cortical thinning in the insular cortex. Microscopically, there was marked capillary proliferation in the substantia nigra, periaqueductal area, putamen, caudate, and medial thalamus (Figure 3). The endothelial cells were increased in number and size. There was a relative sparing of neurons and pallor of the background parenchyma in the substantia nigra (Figure 4). There was no loss of the background parenchyma or cystic change. Only minimal astrogliosis was found. The insular cortex demonstrated pallor and capillary proliferation without good preservation of neurons. There was vacuolization of compacted white matter tracts. This was prominent in the crossing of the superior cerebellar peduncle and medial lemniscus. There were a few vacuoles in the optic chiasm. The location of the lesions, vascular proliferation, and relative sparing of neurons were similar to what is found with subacute necrotizing encephalopathy (Leigh disease).

METHODS

Loading with methionine and isoleucine was carried out in the General Clinical Research Center after the patient fasted for at least 4 hours. The dose of each was 100 mg/kg by mouth. Urine was obtained prior to and at 4 and 8 hours after the administration of the amino acid. Ethylmalonic acid concentration was determined by organic acid analysis performed as described by Hoffmann et al.8

RESULTS

The excretion levels of ethylmalonic acid following loading with isoleucine and methionine is presented in the Table. Isoleucine loading was performed on 2 occasions because of consideration of the pathway by which isoleucine might be a precursor4 and because of the report by Malgorzata et al9 in which isoleucine appeared to be a precursor. However, on neither occasion did the excretion of ethylmalonic acid increase appreciably following isoleucine loading. On the second occasion, the plasma concentration of isoleucine rose from 0.62 mg/dL (47 µmol/L) to 6.28 mg/dL (479 µmol/L) 4 hours after the load. Alloisoleucine was not detected following isoleucine loading. On this occasion, the baseline urine was a 24-hour collection. Following isoleucine loading, the excretion levels of methylsuccinic acid were 28 mmol/mol of creatine, 29 mmol/mol of creatine, and 24 mmol/mol of creatine on the first load and increased from 19 mmol/mol of creatine to 32 mmol/mol of creatine on the second. 2-Methylbutyrylglycine levels were 20 mmol/mol of creatine and 25 mmol/mol of creatinine after isoleucine loading and 4 mmol/mol of creatinine after methionine loading. Isobutyrylglycine levels were 12 mmol/mol of creatine and 25 mmol/mol of creatinine before and after methionine loading, respectively. The serum concentration of lactic acid decreased from 21.4 mg/dL (2.4 mmol/L) to 17.6 mg/dL (2.0 mmol/L) after isoleucine loading and from 25.7 mg/dL (2.9 mmol/L) to 35.3 mg/dL (3.9 mmol/L) after methionine loading.

Following the methionine load, the excretion of ethylmalonic acid rose 1.7 times to a concentration of 648 mmol/mol of creatinine. Furthermore, the excretion of ethylmalonic acid was measured 8 times across 50 days, and the values ranged from 224 mmol/mol of creatinine.

Excretion Levels of Ethylmalonic Acid Following Isoleucine and Methionine Loading*

<table>
<thead>
<tr>
<th>Time, h</th>
<th>Isoleucine</th>
<th>Methionine</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>323</td>
<td>224</td>
</tr>
<tr>
<td>4</td>
<td>348</td>
<td>648</td>
</tr>
</tbody>
</table>

*Values are expressed in millimoles per mole of creatinine.

Figure 3. This high-power view of the caudate nucleus demonstrates the marked endothelial proliferation of capillaries and the increase in the number of capillaries (hematoxylin-eosin, original magnification ×500).

Figure 4. This low-power view of the substantia nigra demonstrates an area of pallor. A healthy portion of the substantia nigra is seen in the right portion of the pallor (hematoxylin-eosin, original magnification ×250).

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to 384 mmol/mol of creatinine, indicating the value after methionine loading to be quite unusual. In addition, the concentration of methylsuccinic acid, which did not change following isoleucine loading and ranged generally from 21 mmol/mol of creatinine to 38 mmol/mol of creatinine, rose to 65 mmol/mol of creatinine following methionine loading. The concentration of methionine in serum had risen from 0.3 mg/dL (20 µmol/L) to 7.7 mg/dL (520 µmol/L) 4 hours after the methionine load. The urinary methionine level was 60 mmol/mol of creatinine 4 hours after the methionine load. A load of 0.5 g/kg of medium-chain triglyceride was followed, as expected, by a rise in ethylmalonic acid excretion from 280 mmol/mol of creatinine to 377 mmol/mol of creatinine. Both parents were given methionine loads. There was no detectable ethylmalonic acid in their urine before or after methionine loading.

Following the results of methionine loading, the patient was treated with a diet restricted in methionine. She was given a mixture of Similac (Ross; Columbus, Ohio), Propimex (Ross), and Moducal (Mead Johnson; Evansville, Ind), providing 111 cal/kg at a weight of 7.8 kg, 0.8 g/kg of whole protein, and 20.5 g/kg of methionine. Solid foods were selected from a list to provide no more than 10 mg of methionine per day. For a healthy infant, a diet composed of Similac would provide 69 mg/kg per day of methionine. The excretion of ethylmalonic acid decreased with this regimen to 186 mmol/mol of creatinine. Her methylsuccinic acid level was 15 mmol/mol of creatinine. Baseline preloading urinary ethylmalonic acid levels had ranged in the month preceding the diet from 224 mmol/mol of creatinine to 367 mmol/mol of creatinine. The parents reported increased alertness and beginning vocalization. Concentrations of serum lactic acid and bicarbonate became normal. The patient developed an acute febrile illness diagnosed as sinusitis and died at 11 months of age.

**COMMENT**

A relationship between this syndrome of ethylmalonic aciduria and the metabolism of sulfur amino acids has been proposed by Duran et al., who found increased excretion levels of inorganic thiosulfite and an absence of detectable sulfite. They also reported 2 sulfur-containing acidic amino acids S-sulfocysteine and S-sulfinocysteine, each of which can be formed nonenzymatically from thiosulfate and cysteine. The increase in excretion of ethylmalonic acid in our patient following methionine loading is consistent with these observations. The mechanism and the site of the defect are not clear.

Methionine is converted normally to homoserine and cysteine. Homoserine is converted to 2-oxobutyric acid, which could be a source of ethylmalonic acid. Cysteine is converted to 2-mercaptopyruvic acid, which is metabolized to pyruvic acid, thiosulfate, and ultimately sulfate. Ethylmalonic acid can be formed via carboxylation of butyric acid, and this appears to be the source of ethylmalonic acid found in short-chain acyl-coenzyme A dehydrogenase deficiency and in multiple acyl-coenzyme A dehydrogenase deficiency. In our patient, loading with a medium-chain triglyceride did not greatly increase the excretion of ethylmalonic acid. Ethylmalonic acid could be a product of isoleucine metabolism after racemization of 2-oxo-3-methylvaleric acid, the precursor of alloisoleucine, from the S to the R form, which is then convertible to 2-methylbutyryl-coenzyme A, 2-ethyl-3-hydroxypropionyl-coenzyme A, and ethylmalonic semialdehyde and to ethylmalonic acid. In our patient, loading with isoleucine did not change the excretion of ethylmalonic acid.

Elevated excretion of ethylmalonic acid and methylsuccinic acid after a load of isoleucine was reported by Malgorzata et al. In these studies, the patient was given 150 mg/kg of isoleucine following which they observed accumulation of 2-methylbutyrylglycine and levels of tiglylglycine lower than in control subjects, but the level of 2-methylbutyrylglycine exceeded 2 SDs above the control mean only slightly and only on 1 time point and the tiglylglycine was quite close to the 2 SDs line for the control subjects. Furthermore, the increase in ethylmalonic acid levels in the urine is difficult to evaluate because the response to isoleucine loading was given in micromoles per liter, whereas the diagnostic level was given in more conventional millimoles per mole of creatinine. In our patient, 2-methylbutyrylglycine was found in small amounts in the urine only after isoleucine loading. Malgorzata et al postulated a block at the levels of 2-methylbutyryl-coenzyme A dehydrogenase; however, the activity of this enzyme was studied and found to be normal. Furthermore, Ots and al found the oxidation of 14C-isoleucine to 14CO2 to be normal in fibroblasts derived from typical patients. In these studies, the oxidation of 14C-butryrate was normal, consistent with our findings on triglyglycine loading. Normal oxidation of fatty acids was also observed by Burlina et al. It is not clear why results were different following isoleucine administration in our patient and the patient of Malgorzata et al, but neither of the siblings reported by their group had petechiae, so they could have been studying a different disease.

A relationship between ethylmalonic aciduria and defective mitochondrial electron transport has been raised by a number of investigators, but the phenotype of these patients appears quite distinct from the ethylmalonic encephalopathy with petechiae syndrome. Hoffmann et al reported a fatal progressive pancytopenia with ethylmalonic aciduria in which there were paracrystalline inclusions in electronmicroscopy findings and reduced activity of cytochrome c oxidase and reductase in muscle. Progressive neurologic disease and partial deficiency of cytochrome oxidase were reported by Lehnert and Ruitenbeek, neither patient had petechiae. A third patient they described who had hematuria had conflicting results on cytochrome c oxidase activity.

A number of typical patients with this syndrome have been found to have normal cytochrome c oxidase activity in fibroblasts. On the other hand, Garavaglia et al reported studies on 2 patients with the typical ethylmalonic encephalopathy with petechiae syndrome in whom cytochrome oxidase activity in fibroblasts was normal but in whose muscles it ranged from 7% to 33% of control. The activity of short-chain acyl-coenzyme A dehydrogenase in fibroblasts was normal. Normal alleles...
for the short-chain acyl-coenzyme A dehydrogenase gene were reported by Nowaczyk et al.11 Another patient with cytochrome oxidase deficiency had a pattern of urinary organic acids dominated by ethylmalonic acid but entirely consistent in pattern with multiple acyl-coenzyme A dehydrogenase deficiency.17 However, in contrast to cells from patients with multiple acyl-coenzyme A dehydrogenase deficiency, fibroblasts from this patient displayed normal oxidation of myristic acid. The clinical manifestations of patients with this syndrome are clearly very different from those usually associated with cytochrome c oxidase deficiency, and certainly most patients with cytochrome c oxidase deficiency do not have ethylmalonic aciduria.13,14

The brother of our proband, case 2, doubtless had the same disease as his sister. Lesions in the brain were suggestive of subacute necrotizing encephalopathy; however, the typical dropout of the background parenchyma seen in Leigh disease, which creates a partially cystic lesion, was not present, and there was vacuolization of compacted white matter tracts, which is not typical of Leigh disease. Vacuolization or spongiouosus in white matter tracts has been documented in aminoacidemias such as nonketotic hyperglycinemia. To our knowledge, case 2 represents the first patient with ethylmalonic encephalopathy in whom an autopsy has been performed.

Results from previous studies to elucidate the bleeding in this disease have been normal. Biopsy results of the petechiae revealed only hemorrhage.3 Findings from studies of prothrombin time, partial thromboplastin time fibrinogen levels, bleeding time, and platelet counts have been normal. Studies of platelet aggregation were normal.3 Tortuous retinal vessels have been correlated with myocardial infarction and deep venous thrombosis. Tissue plasminogen activator is a fibrinolytic agent that is used in the treatment of thrombus.3

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