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Short Communication

Organic Aciduria in Late-onset Biotin-responsive Multiple Carboxylase Deficiency

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Late-onset, biotin-responsive, multiple carboxylase deficiency (McKusick 21021) is an inherited autosomal recessive disorder. The major symptoms of the disease are alopecia skin rash, seizures, ataxia, hypotonia and developmental delay, which typically appear at about 6 months of age, with a range from 3 weeks to 24 months (Wolf *et al.*, 1983b). The primary biochemical defect in this disease was identified as deficient activity of the enzyme biotinidase (biotin-amide amidohydrolase, EC 3.5.1.12; Wolf *et al.*, 1983c). This enzyme catalyzes the removal of biotin from the ε-amino group of a lysine side chain of proteolytic degradation products of the carboxylase holoenzyme, thereby regenerating biotin for reutilization. In biotinidase-deficient patients this biotin-salvage pathway is not operative, and high concentrations of dietary biotin are thus required to prevent the symptoms of the biotin-deficient state (Wolf *et al.*, 1983a).

Organic aciduria is a very general, but surprisingly not a consistent, feature of late-onset multiple carboxylase deficiency. Analysis of the urine of most of these patients have shown the presence of various organic acids associated with the catabolic pathways of the branched chain amino acids (Cowan *et al.*, 1979), whereas pathological amounts of some organic acids only appeared in urine of other patients after a high protein diet (Munnich *et al.*, 1981). In some patients no organic aciduria could be detected (Swick and Kien, 1983; Wolf *et al.*, 1983b).

In this communication we present results of a new case of late-onset multiple carboxylase deficiency. The clinical features were more pronounced than those usually found for individual cases of the disease.

CASE HISTORY

Z.C. is the first child of healthy non-consanguineous parents. Her mother suffered from a resistant diarrhoea during the pregnancy. The delivery and perinatal period were uncomplicated, and she developed normally for the first 10 weeks of life. The onset of resistant myoclonic seizures heralded a relentless downhill course. She lost all social responses and ceased to react to visual or auditory stimuli. She became severely hypotonic and had few voluntary movements. Repeated respiratory infections were troublesome. She developed alopecia, a maculopapular rash and keratoconjunctivitis. Her urine had a pungent feline-like odour. The patient was seen by us at the age of 7 months. At that time she was unresponsive, apart from a startle to loud noise and withdrawal response to pain. The fundi were normal. She had hepatosplenomegaly. Computed tomography showed cerebral atrophy and low attenuation of the white matter. The background activity of the EEG was normal, but epileptiform activity was present over both hemispheres. ERG was normal. VER showed no response and brainstem auditory evoked potential was absent up to 90 dB stimulation. Blood samples for extensive analysis were collected in the fasting state and hourly for 3 h after a load of 1 g protein (kg body weight)$^{-1}$. The plasma pyruvate concentration increased from 135 μmol l^{-1} in the fasting state to a maximum of 241 μmol l^{-1} at 2 h after the protein load. Likewise the serum lactate increased from 7.5 mmol l^{-1} to 8 mmol l^{-1} at 1 h after the protein load. The values for the blood ammonia were 73.8, 79.6 and 51.8 μmol l^{-1}, respectively in the fasting state and 1 and 2 h after the protein load. There was an increased anion gap and a compensated metabolic acidosis. Analysis of the urinary organic acids helped to establish the diagnosis of multiple carboxylase deficiency.

Treatment with biotin (10 mg day^{-1}) was initiated, and increased within 2 weeks to 20 mg day^{-1}. Six weeks after commencing therapy the patient was re-examined. There was a dramatic clinical improvement. She responded socially. Head control, flexor and tensor tone had improved markedly and she was able to sit unsupported. She was fit-free and off antiepileptic medication. The rash, conjunctivitis and respiratory infections had cleared. The EEG and VER had normalized.

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METHODS AND RESULTS

The organic acids were extracted with ethyl acetate and diethyl ether from 1.0 ml acidified urine according to standard procedures. TMS derivatives of the extracted organic acids were analyzed by capillary column (25 m × 0.25 mm, SE 30) gas chromatography. Organic acids were identified by comparing the MU values with authentic standards and by mass spectrometry (GLC–MS) using a Ribermag R10-10 (Rueil Malmaison, France) quadrupole mass spectrometer, combined with a Digital (DEC, Maynard, MA, USA) PDP-11/23 minicomputer. A quantitative determination of urinary organic acids prior to biotin treatment is shown in Table 1. The increased lactic acid content is associated with a deficiency in pyruvate carboxylase (EC 4.6.1.1), while the presence of succinic acid, fumaric acid and 2-ketoglutaric acids is regarded as a secondary response to the deficiency in this enzyme (Van Biervliet *et al.*, 1977). Only 3-hydroxyisovaleric acid and trace amounts to 3-methylcrotonylglycine could be related to a deficiency in the activity of 3-methylcrotonyl-CoA carboxylase (EC 4.6.1.4). No 3-methylcrotonic acid could be detected. The presence of 3-hydroxypropionic acid and low concentrations of methylcitric acid indicated a deficiency in propionyl-CoA carboxylase (EC 4.6.1.3). After treatment with 10 mg biotin day^{-1}, lactic acid and 3-hydroxyisovaleric acid at respective concentrations of 2.3 and 0.33 mmol $(g\ creatinine)^{-1}$ were still present. After treatment with 20 mg biotin day^{-1} the organic acids associated with the disease were absent, and a gas chromatogram of the normal state was obtained.

Table 1 Concentration of the organic acids, diagnostic for multiple carboxylase deficiency, in a urine sample of the patient prior to biotin treatment

Organic acid	*Concentration (mmol (g creatinine)$^{-1}$)*
Lactic acid	8.4
Succinic acid	1.6
Fumaric acid	2.1
2-Ketoglutaric acid	7.3
3-Hydroxyisovaleric acid	17.2
3-Methylcrotonic acid	Not detectable
3-Methylcrotonylglycine	Trace
3-Hydroxypropionic acid	0.7
Methylcitric acid	Trace

DISCUSSION

The clinical presentation and biochemical characteristics of the patient studied were suggestive of late-onset multiple carboxylase deficiency due to biotinidase deficiency. The phenotypical presentation of this disease is variable (Wolf *et al.*, 1983b). The variety and extent of clinical symptoms of our patient exceed those normally found for a single affected individual, and include the less frequently found symptoms like conjunctivitis and hearing loss. Moreover, the pattern of organic acids resembled those of other cases, like increased lactic and 3-hydroxyisovaleric aciduria. On the other hand, very low concentrations of 3-methylcrotonylglycine, methylcitrate and 3-hydroxypropionate were present, and no 3-methylcrotonic acid was detectable. This implies that multiple carboxylase deficiency might remain undetected in a given patient. Oral loading with extra protein will provoke the excretion of the typical metabolites and might be useful in patients that are clinically suggestive of multiple carboxylase deficiency, lacking the chemical characteristics. Our observations finally indicate that clinical and genetic heterogeneity will probably also be a characteristic of multiple carboxylase deficiency, and demonstrate once more the variability in the presentation of inborn errors of metabolism.

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