J. Inher. Metab. Dis. 19 (1996) 739–742

Late-onset holocarboxylase synthetase deficiency

K. M. GIBSON[1,2]*, M. J. BENNETT[3], W. L. NYHAN[4] and C. E. MIZE[5]
[1]*Institute for Metabolic Disease, Baylor Research Institute and Baylor University Medical Center, Dallas, Texas;* [2]*Department of Neurology;* [3]*Department of Pathology, University of Texas Southwestern Medical Center, Dallas, Texas;* [4]*Division of Biochemical Genetics, Department of Pediatrics, University of California, San Diego, La Jolla, California;* [5]*Department of Pediatrics, University of Texas Southwestern Medical Center, Dallas, Texas, USA*

**Correspondence: Baylor Research Institute, 3812 Elm Street, Dallas, TX 75226, USA*

MS received 16.5.96 Accepted 1.7.96

Summary: We report a 21-month-old female patient whose urine organic acid profile suggested a biotin utilization abnormality consistent with multiple carboxylase deficiency. For most previously reported patients, holocarboxylase synthetase deficiency has correlated with the early-onset variant of multiple carboxylase deficiency; conversely, biotinidase deficiency has been characteristic of the late-onset form. *In vitro* enzyme studies revealed that our patient suffered from holocarboxylase synthetase deficiency. We suggest that holocarboxylase synthetase deficiency should be considered in the differential diagnosis of older patients in whom there is suspicion of a defect in biotin metabolism.

Holocarboxylase synthetase (HCS; McKusick 253270) catalyses the covalent attachment of biotin to inactive apocarboxylases to form active holocarboxylases, enzymes that catalyse important reactions in intermediary metabolism. Deficiency of HCS has been reported in more than 20 patients (Fuchshuber et al 1993; Dabbagh et al 1994; Livne et al 1994; Wolf 1995). Key clinical and biochemical findings in HCS-deficient patients include lethargy, irritability, skin lesions, alopecia, episodic ketoacidosis, and a characteristic pattern of organic aciduria. HCS-deficient patients have first presented from a few hours after birth up to 6 years of age; the majority have presented before 3 months of age. To date, all HCS-deficient patients have responded clinically to therapy with biotin (Wolf 1995). HCS deficiency can readily be detected by return to normality of carboxylase activities in cells subcultured in high concentrations of biotin. We report a patient with late-onset HCS deficiency who first presented at 21 months of age.

CASE REPORT AND METHODS

Patient C.H. presented at the age of 21 months with a 12-h history of agitation and lethargy that progressed to obtundation. Growth and development were normal; family history was unremarkable; and there was no recognized preceding illness. She normally ate at 5–6 h intervals, including frequent grape juice, but ate poorly the day preceding hospital admission. On presentation, she was quite somnolent, but arousable (to moderately painful stimuli) and then capable of answering simple questions. Her cry was weak. She was afebrile, with a strong regular pulse (123–142 beats/min), unlaboured respiration (36–38 breaths/min), a blood pressure of 98/45 mmHg and brisk capillary refill time. Her physical examination was remarkable for scattered maculopapular erythematous lesions over her torso and face. The liver was palpable at 3 cm below the right costal margin.

Initial clinical chemistry of the blood revealed the following abnormalities: hypoglycaemia (blood glucose 1.28 mmol/L), uric acid 0.83 mmol/L, 3-hydroxybutyrate 4.4 mmol/L, lactate 7.1 mmol/L, alanine 0.51 mmol/L and ammonia 155 μmol/L. There was a metabolic acidosis (pH 6.91, base deficit −27). The WBC was 26 000/mm^3. Sequential free carnitine:short-chain acylcarnitine concentrations (μmol/L) were (t=number of weeks following presentation) 11:13 (t=0, oral carnitine initiated); 21:41 (t=3, biotin initiated); 74:15 (t=7); 66:5 (t=11); and 67:1 (t=22). Analysis of urine organic acids revealed (mmol/mol creatinine): lactic acid >1000 (control 0–25); 3-hydroxybutyric acid 500 (control 0–3); 3-methylcrotonylglycine 106 (control <2); 3-hydroxyisovaleric acid 110 (control 0–46); methylcitric acid 19 (control 0–12); and 3-hydroxypropionic acid 71 (control 3–10). This pattern was consistent with multiple carboxylase deficiency. Serum biotinidase activity was normal. Parenteral therapy instituted with bicarbonate (1 meq/kg per h), glucose (190 mg/kg per h), and allopurinol (9 mg/kg per day for 30 h) for her acidosis, hypoglycaemia and hyperuricaemia, respectively, was associated within 48 h with significantly improved laboratory observations (blood glucose 5.5 mmol/L; uric acid 0.40 mmol/L; 3-hydroxybutyrate 1.4 mmol/L; lactate 5.2 mmol/L; ammonia 17 μmol/L; pH 7.47; base deficit +0.2; urine organic acids revealed only 3-methylcrotonylglycine and lactic acid). Her rash noted on admission had faded completely by this time, she was alert and talking normally, and frequent-interval oral intake was successfully instituted without problems. By 3 days after initial therapy, blood glucose ranged from 4.4 to 6.2 mmol/L, with plasma 3-hydroxybutyrate 0.7 mmol/L, lactate 4.0 mmol/L and urate 0.31 mmol/L. Modest lactic acidaemia (occasionally to 5.8 mmol/L) without metabolic acidosis has intermittently occurred even when the patient appeared clinically well, but low-dose oral biotin (3 mg/day), initially started 3 weeks following presentation, was associated with effective and persistent (for at least the succeeding 2 years of current follow-up) disappearance of abnormal organic aciduria, and higher biotin supplements have not been undertaken in tandem with her outpatient therapeutic regimen. This regimen of frequent feedings, together with oral biotin (3 mg/day) and carnitine (100 mg/kg per day), has been associated over 2 years with no recurrence of dermatopathy or other clinical/metabolic laboratory aberrations despite three febrile episodes (pharyngitis, upper respiratory infection and otitis media) within 6 months after initial presentation; she has been without significant illness for the subsequent 18 months of current follow-up.

Urine organic acids, plasma carnitine and mitochondrial carboxylases were determined

Table 1 Fibroblast mitochondrial carboxylase activities (pmol/min per mg protein)

	Low biotin[a]			*High biotin*[a]		
	PCC	*MCC*	*PC*	*PCC*	*MCC*	*PC*
Patient						
Mean*	1088†	119‡	375†	839§	546§	544§
Range	949–1275	42–180	290–475	456–1120	309–752	230–790
Percentage of mean control	51	12	26	95	96	199
Control						
mean±SD	2134±1052	1018±335	1428±1021	887±313	567±205	273±168
range	1127–4276	494–1455	709–4077	503–1274	295–961	129–553

[a]Low biotin=D-MEM medium with 10% fetal bovine serum; high biotin=Opti-MEM medium with 4% fetal bovine serum. In low biotin medium, replicate assays (1–3 times) were performed on 10 cell lines from different control subjects, and 4 assays (replicates on same cell line) on patient fibroblasts were performed. In high-biotin medium, replicate assays (2–3 times) were performed on 7 cell lines from different control subjects, and 3 assays (replicates on same cell line) on patient fibroblasts were performed

*Significance determined by Student's one-tailed *t*-test: †$p<0.005$, ‡$p<0.0005$ compared to parallel control; §not significantly different from parallel control

in fibroblast extracts as described (Livne et al 1994). Plasma acylcarnitines were measured by fast atom bombardment–tandem mass spectrometry. For carboxylase assays, low-biotin medium consisted of Dulbecco's modified Eagle's medium (GIBCO-BRL) (DMEM) with 10% fetal bovine serum (FBS; 2.7 nmol/L biotin), while high-biotin medium consisted of Opti-MEM reduced serum medium (GIBCO-BRL) with 4% FBS (440 nmol/L biotin).

RESULTS AND DISCUSSION

The organic acid pattern in the urine of our patient was typical of multiple carboxylase deficiency. The degrees of lactic acidosis and ketosis were consistent with the onset of acute illness. Plasma carnitine and sequential acylcarnitine data were obtained before and after treatment with oral carnitine (100 mg/kg per day) and biotin (3 mg/day). Treatment with carnitine restored the plasma free carnitine to normal and increased the acylcarnitine excretion. Treatment with biotin starting at 3 weeks after presentation led to normal values for carnitine and carnitine ester. Mitochondrial carboxylase activities in fibroblasts derived from the patient are displayed in Table 1. In low-biotin medium, patient fibroblasts demonstrated 51% of control propionyl-CoA carboxylase (PCC) activity, 12% of control 3-methylcrotonyl-CoA carboxylase (MCC) activity, and 26% of control pyruvate carboxylase (PC) activity. All of the carboxylase activities became normal when the cells were grown in high-biotin medium. All of the control mean carboxylase activities decreased significantly ($p<0.0005$) when cells were grown in Opti-MEM with 4% FBS in comparison to cells maintained in DMEM with 10% FBS. The cause of decreased control carboxylase activities in this medium is presently unclear, but it may explain why the patient's cells did not display a greater increase in activity when supplemented with biotin.

The most common initial clinical symptoms in the 12 patients summarized by Wolf (1995), and seen in other patients (Fuchshuber et al 1993; Livne et al 1994), were respiratory

tachypnoea, dyspnoea, hyperventilation, or grunting respirations. In our patient, respiration was apparently unlaboured despite significant metabolic acidosis. Hypoglycaemia has been reported in other HCS-deficient patients (Dabbagh et al 1994), and was detected in our patient. Hypoglycaemia is a common feature of isolated deficiency of MCC, but it could also reflect the deficiency of PC. PC appeared to be the first carboxylase to lose activity during a brief withdrawal of biotin in a patient with HCS deficiency (Barshop et al 1991), although MCC was the most severely affected carboxylase in our patient. Differential response to oral biotin (10–100 mg/day) has been noted in HCS-deficient patients (Fuchshuber et al 1993; Dabbagh et al 1994; Livne et al 1994; Wolf 1995). In a patient receiving 100 mg/day, biochemical and enzymatic abnormalities did not completely resolve. Organic aciduria in our patient resolved during therapy with 3 mg/day oral biotin.

Increasing experience with HCS-deficient patients suggests that the age of onset can be variable. Our patient presented at 21 months of age, which at first suggested biotinidase deficiency. Another patient whom we investigated presented at 20 months of age (Livne et al 1994). Although HCS-deficient patients have presented up to 6 years of age, the majority have presented before 3 months (Wolf 1995). Increasing numbers of HCS-deficient patients presenting beyond the newborn period suggest that HCS deficiency should be considered in the differential diagnosis of older infants and children, in addition to newborns, who present with rash, acidosis or other features consistent with a defect in biotin utilization.

ACKNOWLEDGEMENT

We thank the group at the Duke University Mass Spectrometry Unit for analysis of carnitines and acylcarnitines in our patient.

REFERENCES

Barshop BA, Yoshida I, Ajami A, et al (1991) Metabolism of 1-^{13}C-propionate *in vivo* in patients with disorders of propionate metabolism. *Pediatr Res* **30**: 15–22.

Dabbagh O, Brismar J, Gascon GG, Ozand PT (1994) The clinical spectrum of biotin-treatable encephalopathies in Saudi Arabia. *Brain Dev* **16**(supplement): 72–80.

Fuchshuber A, Suormala T, Roth B, Duran M, Michalk D, Baumgartner ER (1993) Holocarboxylase synthetase deficiency: early diagnosis and management of a new case. *Eur J Pediatr* **152**: 446–449.

Livne M, Gibson KM, Amir N, Eshel G, Elpeleg ON (1994) Holocarboxylase synthetase deficiency: a treatable metabolic disorder masquerading as cerebral palsy. *J Child Neurol* **9**: 170–172.

Wolf B (1995) Disorders of biotin metabolism. In Scriver CR, Beaudet AL, Sly WS, Valle D, eds. *The Metabolic and Molecular Bases of Inherited Disease*, 7th edn. New York: McGraw-Hill, 3151–3177.