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Short Communication

Cerebrospinal Fluid Organic Acids in Biotinidase Deficiency

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Biotinidase deficiency (McKusick 253260) leads to a progressive deficiency of the vitamin biotin, an essential cofactor for the carboxylation reactions of pyruvate, propionyl-CoA, 3-methylcrotonyl-CoA and acetyl-CoA. This disease usually presents with neurological symptoms such as hypotonia, convulsions and ataxia, while skin rash and alopecia usually appear later. If treatment is delayed, irreversible brain damage such as optic atrophy and neurosensory hearing loss may result. The neurological dysfunction appears to be more severe than that observed in holocarboxylase synthetase (HCS) deficiency, propionic acidaemia or isolated 3-methylcrotonyl-CoA carboxylase (MCC) deficiency. A study of the CSF organic acid profile is thus relevant. To date only a few reports on this topic have appeared, demonstrating unusually high CSF lactate levels (Di Rocco et al 1984; Diamantopoulos et al 1986; Fois et al 1986). Here we report detailed CSF organic acid analyses in a series of patients with biotinidase deficiency that are compared with those obtained in HCS and isolated MCC deficiency.

PATIENTS AND METHODS

Seven patients with biotinidase deficiency were included in this study, their ages ranging from 2 weeks to 15 years. All were studied at the time of diagnosis before biotin supplementation. Their clinical condition varied considerably. The youngest patients, discovered by neonatal screening, were either symptom-free (S.M.) or had only mild hypotonia (L.W.) while the older patients were severely ill and had severe neurological abnormalities. The oldest patient (G.D.) presented with acute vision loss at the age of 10 years (Ramaekers et al 1992). For comparison we had the opportunity of studying a patient with isolated MCC deficiency (K.K.) (Baumgartner et al 1991) who had feeding problems, psychomotor retardation and seizures with consecutive hemiplegia at 16 months, and a patient with HCS deficiency (M.T.) who was diagnosed after his second ketoacidotic crisis at 18 months.

Organic acids in CSF and plasma were analysed by gas chromatography–mass spectrometry of the trimethylsilyl derivatives mainly as described earlier (Duran et al 1988). The reference compound 3-hydroxyisovaleric acid (3-HIVA) (silver salt) was synthesized in our laboratory.

RESULTS AND DISCUSSION

The normal organic acid profile of CSF reveals a large amount of lactate (< 2 mmol/L), smaller quantities of 2-hydroxybutyrate and citrate (< 100 μmol/L) and only trace amounts of other organic acids (Coude and Kamoun 1992). Biotinidase-deficient patients accumulate lactate because of functional pyruvate carboxylase deficiency, 3-HIVA due to MCC deficiency and, to a much lesser degree, 3-hydroxypropionate reflecting propionyl-CoA carboxylase deficiency (Table 1). The levels of both lactate and 3-HIVA found in our patients correlate with the severity of clinical symptoms and reflect the degree of biotin deficiency. However, the oldest patient, G.D., had normal lactate levels that could be explained by the lower gluconeogenic demand in older subjects. It should be noted that this unusual patient showed biphasic kinetics of biotinidase with one normal K_m (with very low V_{max}) and one highly elevated K_m for biocytin. The CSF/plasma ratio of lactate in this patient was only moderately increased at 1.9, whereas the other patients had ratios of 2.7–3.7. Increased CSF lactate has been shown to cause cerebral acidosis (Diamantopoulos et al 1986) leading to hyperventilation.

As well as lactate, higher levels of 3-HIVA were found in CSF compared to those in plasma, except in the youngest asymptomatic patient. The CSF/plasma ratios of 3-HIVA in three symptomatic patients ranged between 4.3 and 8.4. Interestingly, 3-HIVA concentration in CSF and its CSF/plasma ratio (27.8) were highly elevated in the 15-year-old child who had normal lactate levels and only slightly elevated 3-hydroxypropionate. Since this patient suffered from severe chronic neurological problems (optic atrophy, spastic paraparesis), we speculate that 3-HIVA may have a specifically harmful effect on the brain. No major changes were observed for other organic acids in the CSF such as 2-hydroxybutyrate and citrate (Table 1).

Biotinidase deficiency results in progressive loss of biotin from birth onwards with concomitant decrease of the activities of the biotin-dependent carboxylases, particularly of the mitochondrial ones. We have shown in a lethal case that carboxylases were much more severely decreased in brain than in liver and kidney (Baumgartner et al 1989). The increased CSF/plasma ratio of lactate and 3-HIVA in biotinidase-deficient patients provides further evidence for our idea that biotin depletion occurs earlier in the CNS than in other organs. This is also in accordance with the presence of neurological symptoms in the absence of significant organic aciduria seen in several cases.

Treatment with biotin rapidly reversed all CSF organic acid abnormalities (results not shown), indicating an efficient transfer of pharmacological doses of biotin across the blood–brain barrier. The analysis of CSF organic acids is an important and sensitive parameter for the evaluation of biotin status in brain. It may also be of prognostic value. These studies provide the basis for a possible role for *in vivo* magnetic resonance spectroscopy of brain lactate for the monitoring of treatment.

Comparison of the organic acid abnormalities seen in biotinidase deficiency with the other carboxylation disorders showed interesting differences. Neither the isolated MCC-deficient nor the HCS-deficient patient had strikingly increased CSF/plasma ratios of lactate or 3-HIVA. However, the CSF concentration of 3-HIVA in patient K.K. with MCC deficiency was as high as in the severely ill patients with biotinidase

Table 1 Concentrations of organic acids (μmol/L) in the CSF and plasma of 7 patients with biotinidase deficiency, 1 patient with isolated 3-methylcrotonyl-CoA carboxylase (MCC) deficiency and 1 patient with holocarboxylase synthetase (HCS) deficiency

Patient	*Age* (months)	*Lactate, CSF/plasma*	*3-OH-isovalerate, CSF/plasma*	*3-OH-propionate, CSF*	*2-OH-butyrate, CSF*	*3-OH-butyrate, CSF*	*Citrate, CSF*
S.M.	0.5	2283/5990[a]	12/13	ND	31	30	64
L.W.	2	3811/3918	104/24	ND	46	18	77
M.S.	3	5061/NA	215/NA	ND	74	Trace	Trace
N.B.	4	12535/3829	278/33	ND	253	153	Trace
M.R.	11	8053/2200	297/NA	ND	161	67	100
T.S.	22	7343/2723	211/46	ND	394	882	167
G.D.	180	1749/974	250/9	5	52	7	Trace
K.K. (MCC)	16	830/1385	285/166	ND	17	?	124
M.T. (HCS)[b]	18	2660/4063	83/87	14	76	57	113
Controls		< 1800/< 2000	< 2/< 2	ND	< 80	< 60	< 100

ND = not detectable
NA = not available
[a]Artefact of blood sampling
[b]Further metabolites found in CSF of only this patient were 3-hydroxy-n-valerate and 3-methylcrotonylglycine

deficiency (Table 1). Indeed, this patient had severe neurological abnormalities. In contrast, the HCS-deficient patient (M.T.), whose CSF 3-HIVA was lower than in any of the symptomatic patients with biotinidase deficiency, showed no neurological symptoms while his lymphocyte carboxylase activities were severely decreased (< 10% of mean normal). These findings indicate that in HCS deficiency the brain carboxylases are not predominantly affected in contrast to biotinidase deficiency.

Patients with defects in which a cerebral accumulation of C-4 or C-5 hydroxycarboxylic acids occurs are prone to neurological dysfunction. This holds true not only for biotinidase deficiency, but also for 4-hydroxybutyric aciduria and L-2-hydroxyglutaric aciduria. More investigations are needed for the elucidation of the effect of these acids on brain function.

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REFERENCES

Baumgartner ER, Suormala TM, Wick H et al (1989) Biotinidase deficiency: A cause of subacute necrotizing encephalomyelopathy (Leigh Syndrome). Report of a case with lethal outcome. *Pediatr Res* **26**: 206–266.

Baumgartner ER, Suormala TM, Wiesmann U, Pegel M, Niederhoff H, Lehnert W (1991) Isolated biotin-resistant beta-methylcrotonyl-CoA carboxylase deficiency: a severe disease. *Abstracts 29th Annual SSIEM Symposium* p. 130.

Coude M, Kamoun P (1992) Organic acids in post-mortem cerebrospinal fluid. *Clin Chim Acta* **206**: 201–206.

Diamantopoulos N, Painter MJ, Wolf B, Heard GS, Roe C (1986) Biotinidase deficiency: accumulation of lactate in the brain and response to biotin therapy. *Neurology* **36**: 1107–1107.

Di Rocco M, Superti-Furga A, Durand P et al (1984) Different organic acid patterns in urine and in cerebrospinal fluid in a patient with biotinidase deficiency. *J Inher Metab Dis* **7** (Suppl 2): 119–120.

Duran M, Bruinvis L, Ketting D, de Klerk JBC, Wadman SK (1988) *Cis*-4-decenoic acid in plasma: a characteristic metabolite in medium-chain acyl-CoA dehydrogenase deficiency. *Clin Chem* **34**: 548–551.

Fois A, Cioni M, Balestri P, Bartalini G, Baumgartner R, Bachmann C (1986) Biotinidase deficiency: metabolites in CSF. *J Inher Metab Dis* **9**: 284–285.

Raenmaekers VT, Suormala TM, Brab M, Duran M, Heiman G, Baumgartner E (1992) A biotinidase K_m variant causing late onset bilateral optic neuropathy. *Arch Dis Child* **67**: 115–119.