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Late-onset holocarboxylase synthetase-deficiency: pre- and post-natal diagnosis and evaluation of effectiveness of antenatal biotin therapy

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Abstract The clinical and biochemical findings in a family with late-onset holocarboxylase synthetase (HCS) deficiency are described. The index patient had two life-threatening episodes of metabolic decompensation at the age of 13 and 18 months with ketotic hypoglycaemia, vomiting and progressive loss of consciousness. The child recovered without biotin therapy. Organic aciduria characteristic of multiple carboxylase deficiency (MCD) was found, however, the key metabolites were only slightly elevated in some samples. Biotinidase deficiency was considered but excluded by the finding of normal plasma biotinidase activity. The correct diagnosis was made only at the age of 19 months when severe MCD was found in lymphocytes in the presence of normal plasma biotin concentration. HCS deficiency was confirmed by fibroblast studies. Biotin therapy (20 or 40 mg/day) prevented further episodes and normalized biochemical parameters with so far normal development.

During two subsequent pregnancies, 10 mg biotin/day was administered to the mother from the 20th week of gestation. At delivery plasma biotin in cord blood samples was 3–4 times higher than in maternal plasma. The 2nd child was unaffected. In the 3rd pregnancy prenatal diagnosis was performed at 16 weeks of gestation. The concentration of methylcitrate in amniotic fluid was within the normal range and that of 3-hydroxyisovalerate only slightly elevated. However, enzyme assays in cultured amniotic fluid cells were consistent with an affected fetus. At birth, carboxylase activities in lymphocytes of this newborn were only moderately decreased to 37% of mean normal. HCS deficiency was confirmed postnatally in fibroblasts. Development remains normal on biotin therapy (20 mg/day).

Conclusion Prenatal diagnosis in families with milder forms of HCS deficiency has to be performed by enzyme assays in cultured amniotic cells since organic acid analysis of amniotic fluid may be inconclusive in affected fetuses. Biotin administered prenatally is effectively taken up by the fetus and prevents functional deficiency of the carboxylases in an affected newborn.

Key words Holocarboxylase synthetase deficiency · Biotin therapy · Prenatal diagnosis

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Abbreviations *ACC* acetyl-CoA carboxylase · *FCS* fetal calf serum · *HCS* holocarboxylase synthetase · *3-HIVA* 3-hydroxyisovalerate · *MCC* 3-methylcrotonyl-CoA carboxylase · *MCD* multiple carboxylase deficiency · *3-MCG* 3-methylcrotonylglycine · *MeCit* methylcitrate · *PC* pyruvate carboxylase · *PCC* propionyl-CoA carboxylase

Introduction

Holocarboxylase synthetase (HCS) deficiency is a rare inborn error of biotin metabolism. HCS catalyses the covalent binding of biotin to four biotin-dependent enzymes in man: propionyl-CoA carboxylase (PCC, EC 6.4.1.3), 3-methylcrotonyl-CoA carboxylase (MCC, EC 6.4.1.4), pyruvate carboxylase (PC, EC 6.4.1.1) and acetyl-CoA carboxylase (ACC, EC 6.4.1.2). Defective function of HCS leads to multiple deficiency of the carboxylases (MCD) and to severe life-threatening illness [18]. Symptoms include feeding difficulties, vomiting, tachypnoea, seizures, hyper- or hypotonia and progressive loss of consciousness which may lead to coma and death. Although half of reported patients presented within the first days or weeks of life, others presented with acute metabolic derangement later, at the age of 14–20 months [2, 9, 11, 13]. Such late-onset patients cannot be differentiated clinically from patients with biotinidase deficiency, the other known congenital disorder of biotin metabolism also leading to MCD. However, in contrast to patients with biotinidase deficiency all patients with HCS deficiency have suffered from metabolic acidosis, mild hyperammonaemia and typical organic aciduria some time during the course of the disease [18]. Oral treatment with pharmacological doses of biotin reverses the biochemical and clinical abnormalities. However, in some few cases the response is only partial [17, 19]. In all well-documented patients, decreased affinity of HCS for biotin was shown to be the primary defect [2, 3].

We describe clinical and biochemical findings in a patient with late-onset HCS deficiency, as well as the results of prenatal and/or postnatal diagnosis of an unaffected and an affected sibling of the index patient. An aim of the study was to evaluate which diagnostic methods are appropriate for the correct prenatal and postnatal diagnosis of milder forms of HCS deficiency.

Case reports

Index patient

Case 1, a boy, was the first child of healthy non-consanguineous German parents born at term after an uneventful pregnancy and delivery in 1990. Birth weight was 3560 g, length 52 cm and the Apgar score was 10 after 5 and 10 min. Early development was normal but at the age of 3 months a skin rash resembling neurodermitis developed in the absence of other skin abnormalities or hair problems. Statomotor, mental and psychosocial development have always been normal. At the age of 13 months he suffered a life-threatening episode after orchidopexy. Twelve hours after the operation he started with massive vomiting, became progressively acidotic, hypotonic, somnolent and finally unresponsive. Laboratory findings showed hypoglycaemia (1.8 mmol/l), metabolic acidosis (pH 7.05, PCO_2 11.7, BE-26 mmol/l) with an anion gap of 27 mmol/l, hyperammonaemia of 139 μmol/l (reference value <40), hyperlactataemia of 6.39 mmol/l (reference value <2) and massive ketonuria. He recovered well with i.v. fluids without biotin therapy and appeared healthy when discharged. Urinary organic acid analysis revealed elevated excretion of lactate and 3-hydroxyisovalerate (3-HIVA) suggesting multiple carboxylase deficiency (see results). Biotinidase deficiency, considered because of the late onset of the illness, was excluded by normal plasma biotinidase activity. Major causes of hypoglycaemia were excluded by normal results of glucagon and insulin tolerance tests, i.v. fructose loading and cortisol profile. The preliminary diagnosis was ketotic hypoglycaemia.

A second metabolic crisis occurred following a febrile common cold at the age of 18 months. The clinical signs, laboratory findings (glucose 2.2 mmol/l, pH 6.95, BE-29 mmol/l, anion gap 31 mmol/l, lactate 9.7 mmol/l) and the course of illness were similar to those of the first crisis, and the child recovered again without biotin therapy. At the age of 19 months when the child was apparently well, MCD was confirmed in lymphocytes in the presence of normal plasma biotin concentration, indicative of HCS deficiency [17]. Biotin therapy was started with a dose of 2×10 mg/day and increased to 2×20 mg/day at the age of 2 years because of persistence of minor biochemical abnormalities at the lower dose [17]. No further episodes of metabolic decompensation have occurred and development has been normal. The skin rash disappeared although he suffered from bronchial asthma at 6 years of age.

Detailed biochemical studies in case 1 have been reported elsewhere [17 patient TM].

Siblings of case 1

Case 2, a girl, was born in 1993 at term after an uneventful pregnancy and delivery. The mother received supplemental biotin (2×5 mg/day) during the pregnancy from the 20th week of gestation. At birth the child was given oral biotin (2×10 mg/day) which was discontinued after 2 months when enzyme studies in cultured skin fibroblasts revealed that she was unaffected.

Case 3, a boy, was born in 1994. During the pregnancy prenatal diagnosis was requested by the mother and performed by amniocentesis at the 16th week of gestation. Enzyme assays in amniotic fluid cells suggested an affected fetus whereas organic acid concentrations in amniotic fluid were interpreted as normal (see results). Supplemental biotin (2×5 mg/day) was again given to the mother from the 20th week of gestation and to the child immediately after birth (2×10 mg/day). HCS deficiency was confirmed by assay of carboxylase activities both in cord blood lymphocytes and cultured skin fibroblasts (see results). So far no clinical abnormalities have been observed and development has been normal.

Methods

Organic acids were determined in urine by gas chromatography/mass spectrometry [4] and in amniotic fluid by stable isotope dilution analysis [7].

Biotin concentration was determined in plasma and serum by a microbiological assay using *Lactobacillus plantarum* ATCC 8014 [6]

which detects only biotin-d-sulphoxide in addition to biotin. Total biotin concentration in amniotic fluid samples was determined by an avidin-binding assay [15] which detects most biotin derivatives with an intact ureido-ring in addition to biotin.

Lymphocytes were isolated from blood samples and fibroblasts cultured from skin biopsies as described [14]. Fibroblasts were routinely cultured in a standard medium containing 10% fetal calf serum (FCS, Gibco BRL, Life Technologies, UK) which constitutes a biotin concentration of 10^{-8} mol/l. For confirmation of HCS deficiency, carboxylase activities were measured in fibroblasts grown for one subculture (7–14 days) in 3 different media; (1) the standard FCS-based medium; (2) a high-biotin medium prepared by supplementing the standard medium with 10^{-5} mol/l biotin, and (3) a low-biotin medium prepared by replacing FCS with newborn calf serum (NBCS, Gibco BRL, and BIOSPA GmbH, Germany) which resulted in a biotin concentration of 10^{-10} mol/l.

Amniotic fluid cells were routinely grown in a standard medium optimized for this cell type (AmnioMAX-C100 medium, Gibco BRL, Life Technologies, UK). For prenatal diagnosis amniotic fluid cells and fibroblasts (positive and negative controls) were grown to confluency in the appropriate standard media supplemented with 10^{-5} mol/l biotin. The medium was then removed, the cell layers rinsed with Hanks' balanced salt solution (Gibco BRL) and NBCS-based low-biotin medium added. After 4–8 days of further growth carboxylase activities were measured.

The activities of the biotin-dependent mitochondrial carboxylases (PCC, MCC, PC) were assayed in cell homogenates by measuring the incorporation of ^{14}C-bicarbonate into acid-non-volatile products as described [14, 16]. Glutamate dehydrogenase (GLDH) activity was determined as a mitochondrial biotin-independent control enzyme by a NADH linked assay [8]. Protein concentration in homogenates was determined by a modified Lowry method [14].

Results

Biochemical findings in the index case 1

MCD was suspected due to highly elevated concentrations of typical organic acids, i.e. 3-HIVA, 3-methylcrotonylglycine (3-MCG), lactate and methylcitrate (MeCit) in urine obtained during acute episodes. Results of quantitative analyses performed during the second episode at the age of 18 months are shown in Table 1. At the age of 19 months, when the child was apparently well, the organic acid abnormalities were less prominent in all samples, especially in one urine sample which showed only a tenfold elevation of 3-HIVA, slightly elevated lactate and trace amounts of 3-MCG (Table 1). The concentrations of the important metabolites were also increased in plasma and CSF, however, their concentrations were lower in CSF than in plasma [5]. Severe MCD was confirmed in lymphocytes [17]. Biotin therapy resulted in rapid biochemical improvement. Organic acid excretion became normal with 20 mg biotin/day (Table 1) whereas complete normalization of carboxylase activities in lymphocytes required a higher biotin dose of 40 mg/day [17].

Prenatal diagnosis

In amniotic fluid obtained at the 16th week of gestation from the affected pregnancy the concentration of MeCit was within the normal range (0.24 μmol/l, control range: 0.08–0.50, $n = 28$) and that of 3-HIVA only slightly above the control range (7.4 μmol/l, control range: 1.7–5.6, $n = 28$). This result was considered not to indicate an affected fetus since a previous positive case had shown a much higher 3-HIVA concentration of 38.8 μmol/l [7]. In contrast, carboxylase activities in cultured amniotic fluid cells, measured after 5 days biotin deprivation, were severely decreased with even lower levels than those found in fibroblasts of the index patient (Table 2) suggesting an affected fetus. This discrepancy between metabolite levels and enzyme activities cannot be explained by unprescribed biotin supplementation since the total biotin concentration in amniotic fluid was normal (2.2–2.6 nmol/l in 3 determinations, control range: 1.5–2.2, $n = 4$). It should be noted that carboxylase activities were normal using similar culture conditions in control skin fibroblasts and amniotic fluid cells of an unaffected fetus from another family with HCS deficiency (Table 2).

Antenatal and postnatal biotin therapy

The mother received biotin therapy at a dose of 2×5 mg/day from the 20th week of gestation during two pregnancies. Effectiveness of the antenatal biotin therapy was evaluated by measuring carboxylase activities in lymphocytes and/or plasma biotin concentrations at birth. Biotin concentrations in the cord blood plasma of both newborns (cases 2, 3) were similarly elevated and 3–4 times higher than in plasma of the mother at delivery (Table 3). Carboxylase activities in cord blood lymphocytes, measured only in case 3, were decreased to

Table 1 Concentrations of some typical organic acids in urine (mmol/mol creatinine) of the index patient case 1[a]. Where no quantitative analysis was performed the degree of elevation was designated as follows: + + + = massive, + + = strong, + = slight, – = not present (ND not detectable)

Subject	Biotin therapy, presentation	Lactate	3-HIVA	3-MCG	MeCit
Case 1	No therapy, 2nd acute episode	8857	2077	+ + +	+
	No therapy, well-being	970	1180	+ +	137
	No therapy, well-being	64	182	trace	–
	20 mg biotin/day	Normal	11	ND	ND
	40 mg biotin/day	17	14	ND	ND
Controls		29–43	< 18	ND	ND

[a] Some of the values have been presented earlier [17]

Table 2 Prenatal diagnosis of HCS deficiency performed by assaying the level of mitochondrial carboxylase activities in cultured amniotic fluid cells (AC) and skin fibroblasts (FB) as controls. Cells were first grown in high-biotin (10^{-5} mol/l) medium and then for the noted periods in low-biotin (10^{-10} mol/l) medium without subculture

Patient	Cell type	Biotin in medium	Carboxylase activities pmol/min/mg protein			nmol/min/mg protein GLDH
			PCC	MCC	PC	
At risk pregnancy	AC	High	1598	636	1175	40.5
	"	Low for 5 days	132	46.6	98.8	43.4
Unaffected pregnancy	AC	High	1432	291	6811	30.8
	"	Low for 4 days	1143	300	4017	26.8
	"	Low for 8 days	1638	318	4300	32.8
Case 1	FB	High	1019	407	251	27.1
	"	Low for 5 days	688	195	150	23.7
Control cells 1	FB	High	292	209	698	15.3
	"	Low for 5 days	363	216	864	18.3
Control cells 2	FB	High	822	202	2463	24.0
	,,	Low for 5 days	802	205	2522	23.4

Table 3 Carboxylase activities in lymphocytes and/or biotin concentration in plasma in cord blood of the newborns cases 2 and 3 and in blood of the mother at delivery

Subject	Blood sample	Hours after last biotin dose	Plasma biotin nmol/l	Carboxylase activities, pmol/min/mg protein			nmol/min/mg protein GLDH
				PCC	MCC	PC	
Mother	Peripheral	8.0	69.7	–	–	–	–
Case 2	Cord blood		202	–	–	–	–
Mother	Peripheral	8.5	64.5	604	552	42.7	23.3
Case 3	Cord blood		253	260	247	106	20.3
Control values[a] (mean ± SD and range):							
	Cord blood		2.28 ± 0.99	698 ± 167	666 ± 252	288 ± 70	28.8 ± 6.9
			0.97–4.29	400–960	287–1058	180–423	17.4–40.8
	Peripheral		1.80 ± 0.84	568 ± 129	396 ± 128	69.6 ± 23.0	28.9 ± 7.0
			0.65–4.83	283–921	185–824	35.4–147	14.4–48.6

[a] number of different individuals studied were 18 for all analyses in cord blood, 126 for plasma biotin concentrations and 59 for all enzyme activities in peripheral blood

Table 4 Carboxylase activities in cultured skin fibroblasts grown for one passage in media with three different biotin concentrations: high (10^{-5} mol/l), standard (10^{-8} mol/l) and low (10^{-10} mol/l)

Case	Biotin level in medium	Carboxylase activities, pmol/min/mg protein			nmol/min/mg protein GLDH
		PCC	MCC	PC	
1	Low	22.9	4.3	1.5	31.0
	Standard	720	195	225	25.1
	High	1187	598	550	27.4
2	Low	767	314	1370	20.2
	Standard	846	494	1013	21.3
	High	890	526	1248	19.0
3	Low	64.7	19.9	96.9	19.1
	Standard	362	116	510	20.4
	High	647	360	821	22.2
Control values:					
median	Low	628(262–1951)	156(98.0–497)	615(202–1253)	16.6(9.2–36.5)
(range)	standard	717(221–1742)	300(130–738)	701(279–3041)	17.5(7.9–41.8)
n[a] = 21	High	718(202–1806)	306(128–589)	664(268–2557)	20.3(8.4–39.1)

[a] n = number of different cell lines

37% of the mean normal value in spite of the highly elevated biotin concentration in plasma. In the first urine sample of case 3 organic acid concentrations were within the normal range and biotin concentration was highly elevated (1330 nmol/mmol creatinine, control range: 3.4–204, $n = 64$). The findings in lymphocytes were in

accordance with the results obtained in amniocytes suggesting that case 3 suffered from HCS deficiency.

Both cases 2 and 3 received 20 mg biotin/day from birth. In case 2 biotin therapy was stopped later when she was shown to be unaffected. In case 3 biotin therapy was continued and at the age of 7 and 19 months carboxylase activities in lymphocytes and organic acid excretion were normal.

Carboxylase assay in cultured skin fibroblasts

The diagnosis of HCS deficiency was confirmed in the index case 1 and case 3, and excluded in case 2 by assays in cultured skin fibroblasts (Table 4). After one subculture in the low-biotin (10^{-10} mol/l) medium the activities of the mitochondrial biotin-dependent carboxylases were severely decreased in cells of cases 1 and 3 but normal in cells of case 2. In both affected siblings carboxylase activities in fibroblasts grown in the standard FCS-based medium with a moderate biotin concentration of 10^{-8} mol/l (which is 5–10 times the physiological plasma biotin concentration in man) were normal or only mildly affected. In the high-biotin (10^{-5} mol/l) medium the activities were well within the normal range in all cell lines.

Discussion

The results of studies in this family emphasize the need to consider HCS deficiency as well as biotinidase deficiency in patients with late-onset of clinical symptoms and increased excretion of characteristic organic acids. The diagnosis of HCS deficiency in the index patient was clearly demonstrated by the severe MCD in fibroblasts and lymphocytes in the presence of normal plasma biotinidase activity and biotin concentration, and a marked clinical and biochemical improvement on biotin supplementation. Furthermore, detailed studies in fibroblasts, reported elsewhere [17], showed only a tenfold decreased affinity of HCS for biotin in contrast to much greater decreases in early presenting patients [3, 17]. This suggests the presence of a relatively mild Km defect in our patient.

Such a mild defect fits in well with the later onset of symptoms of an episodic nature and recovery without biotin treatment. However, these episodes were severe and life-threatening. The intermittent nature of the disorder is reflected by considerable variation of the degree of organic acid abnormalities. In fact, one urine sample from a period of well-being showed only tenfold elevated 3-HIVA, mildly elevated lactate and trace amounts of 3-MCG. This emphasizes the need to obtain samples during illness for more reliable diagnosis. Also all patients with clearly elevated excretion of 3-HIVA should be investigated for both biotinidase and HCS deficiency.

The results of this study also clearly show that the biotin content of the media must be carefully controlled to detect MCD in cultured cells. In this respect the commonly used media supplemented with FCS have a too high biotin concentration to allow the detection of an abnormality in cases with such a mild form of HCS deficiency as in the present family [17].

The existence of a mild form of HCS deficiency can complicate prenatal diagnosis. As shown here, the concentration of typical organic acids in amniotic fluid may be normal (MeCit) or only slightly increased (3-HIVA) in an affected pregnancy. Therefore prenatal diagnosis must be performed by enzyme assay in cultured fetal cells. Importantly, the severity of the defect is very variable among different families. Therefore, optimal conditions for prenatal diagnosis must be selected for each family individually since culture conditions with respect to biotin concentration of the medium and culture time in low-biotin medium influence the development of MCD in HCS-deficient cells. This study shows that fibroblasts of the index patient can be reliably used for this purpose.

We confirm earlier reports that antenatal biotin given to the mother is effectively transported by the placenta and can prevent severe carboxylase deficiency in an affected newborn [10, 12]. It is known that plasma biotin concentration is higher in cord blood than in the blood of the mother on a normal diet [1]. This is also true during biotin supplementation with pharmacological doses of biotin: plasma biotin was 30-fold normal in the mother and 100-fold normal in cord blood (see Table 3). Furthermore, biotin concentration was highly elevated in urine of the newborn. However, under these conditions carboxylase activities in lymphocytes of the affected newborn were clearly below the normal range inspite of the relatively mild form of HCS deficiency. This finding confirms that the assay of carboxylase activities in lymphocytes is a sensitive parameter for effectiveness of biotin therapy.

Some important aspects of the response to biotin treatment in late onset HCS deficiency are illustrated by this study. In both our patients oral biotin therapy with 20–40 mg/day (1.4–2.5 μg biotin/kg body weight/day) resulted in an excellent biochemical and clinical response in accordance with the late-onset of clinical symptoms and mildly decreased affinity of HCS for biotin found in fibroblasts. Fortunately, the life-threatening episodes of metabolic decompensation in the index patient, from which recovery took place without biotin therapy, seems to have caused no permanent damage. Indeed, under biotin therapy there has been normal development so far up to 6 years of age. Irreversible damage to the CNS, e.g. neurosensory hearing loss, optical nerve atrophy or ataxia, which is seen in patients with biotinidase deficiency after similar episodes are not present. Probably the pathogenesis of these two biotin-responsive disorders is different with respect to the involvement of the CNS [5] resulting in a much better prognosis in treated late-onset HCS deficiency.

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