Medium-chain acyl-CoA dehydrogenase deficiency in two siblings with a Reye-like syndrome

An increasing number of reports indicate that patients with some inherited metabolic diseases may have symptoms resembling those of Reye syndrome. We describe two siblings who developed a Reye-like syndrome at ages 16 and 18 months, respectively, after a viral illness and salicylate therapy. Both had fasting hypoglycemia and hypoketonemia. At the time of the acute episode and after ingestion of a medium-chain triglyceride load, one of them excreted large amounts of abnormal metabolites derived from the ω- and (ω-1)-oxidation of medium-chain fatty acids. Medium-chain acyl-CoA dehydrogenase activity was lower than 20% of control values in fibroblasts from both patients. This enzyme defect should be considered in children with a Reye-like syndrome with these distinctive manifestations. (J PEDIATR 106:918, 1985)

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TWO YOUNG SIBLINGS developed characteristic symptoms of Reye syndrome. A deficiency of medium-chain acyl-CoA dehydrogenase was suspected on the basis of gas chromatographic–mass spectrometric analysis of urine, and was confirmed by in vitro studies of fatty acyl-CoA dehydrogenation in skin fibroblasts.

CASE REPORTS

Patient 1 was born at term in 1980, with birth weight 4050 gm. He had mild hypoglycemia in the immediate postnatal period, then was well for the first year of life. At age 18 months he had a febrile illness with rhinorrhea and sore throat, for which he received 300 mg salicylate daily. Four days later he developed persistent vomiting, and after fasting for 20 hours was found stuporous in his bed at 6:00 AM. At admission he had stage III coma and generalized seizures. Clinical examination yielded negative findings except for temperature 36° C. Blood glucose concentration was 15 mg/dl. Other pertinent laboratory data included hemoglobin 11.4 gm/dl; WBC 10,100/μl, with 43% polymorphonuclear leukocytes, 38% lymphocytes, 15% monocytes, 2% vacuolated basophilic monocytes, and 2% plasmocytes. Serum bilirubin concentration was 4 μmol/L (normal 5 to 18 μmol/L); SGOT was 40 IU/ml, SGPT 37 IU/ml, creatine kinase 17 IU/ml, ammonia 35 μmol/L (normal 40 μmol/L); values for plasma proteins and coagulation factors, pH, and plasma electrolytes were normal. CSF contained <1 cell/μl, protein 0.18 gm/L, and glucose 17 mg/dl. Plasma salicylate concentration was 3.6 mg/dl. The child gradually awakened with intravenous administration of glucose, but the EEG findings were still abnormal 10 hours after admission, with diffuse bilateral slow waves and a spike focus. By the next morning, the infant was fully alert and active with normal EEG findings. By the second hospital day he developed hepatomegaly, and needle percutaneous biopsy of the liver was performed. Two days later the liver returned to normal size. After dietary studies, the patient was discharged from the hospital, his parents being advised to avoid prolonged fasting as well as salicylate therapy. Since then he has had no recurrence of symptoms and is developing normally.

Patient 2 was born at term in 1977 after an uneventful gestation and delivery. Her early course and development were normal. At age 16 months she developed an illness characterized by fever (39° C), rhinorrhea, pharyngitis, right-sided otitis media, and a generalized maculopapular rash. She received salicylate 200 mg/day. Five days later she started vomiting and feeding poorly, and 1 day later was found unarousable in her bed at 8:00 AM. At admission she was in a stuporous state, with otherwise negative

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findings on general examination, temperature 37° C, and normal liver size. Blood glucose concentration was 28 mg/dl, and the urine was slightly positive to nitroprusside testing. Other laboratory data included hemoglobin 11.8 gm/dl; WBC 11,300/μl, with 32% polymorphonuclear leukocytes, 64% lymphocytes, 1% monocytes, and 3% plasmocytes; plasma electrolytes, calcium, pH, and creatinine values were normal. Bilirubin was 6 μmol/L, SGOT 924 IU/ml, SGPT 696 IU/ml, ammonia 77 μmole/L, prothrombin time 77% of normal control, and plasma albumin 34 gm/L; coagulation factors were normal. CSF contained <1 cell/μl, protein 0.17 gm/L, and glucose 15 mg/dl. The hypoglycemia was corrected with intravenously administered glucose therapy. The EEG recorded bilateral diffuse slow waves predominating in the right posterior area of the brain. The patient recovered progressively, and 10 hours after admission was again alert and active; soft, painless hepatomegaly was noted, but disappeared 3 days later. Hb_sAg and Hb_sAb by radioimmunoassay were negative. The diagnosis of Reye syndrome was considered. Three years later, at 5 years of age, the patient was referred to our clinic after her brother's illness. Examination revealed an alert, intelligent girl with normal development; liver size and muscular strength were normal. Since the first episode of hypoglycemic coma, she has had no other incidents, despite the occurrence of several viral infections (mumps and varicella) in the meanwhile.

Patient 3, the oldest sibling, now 5 years of age, never had any similar acute episode, despite various viral illnesses, salicylate ingestion, and fasting. No relatives (the mother has 12 siblings and the father eight) had any evidence of related disease.

METHODS

Dietary studies. After informed consent of the parents was obtained, the affected children and the older sister were fasted for 20 hours after 4 days of a regular diet. Thus, patient 1 was studied in the period after the attack, and patient 2 3 years later. In patient 1 the fast was terminated by the ingestion of 4.5 gm medium-chain triglycerides (Tricème, Sopharga; Paris) containing 65% octanoic acid and 26% decanoic acid.

In vitro studies of fatty acyl-CoA dehydrogenation. The MCADH activity in cultured skin fibroblasts was assayed with octanoyl-CoA and decanoyl-CoA as described previously.[1] The reference enzyme, glutamate dehydrogenase, was measured according to the method of Schmidt.[2]

Analytic procedures. Urine samples were subjected to gas chromatographic–mass spectrometric analysis, and the organic acids were quantified by selected ion monitoring of their protonated molecular ions by ammonia chemical ionization. Plasma dicarboxylic acids were measured by a similar technique, with phenylsuccinic acid as internal standard.[3] Free carnitine and acylcarnitine concentrations were determined in plasma, liver, and urine.[4] Octanoate was extracted from 50 μl plasma samples and measured by selected ion monitoring of the [M-15] fragment of its trimethylsilyl derivative [m/z 201] with itaconic acid as internal standard.

Table I. Urine substrate concentrations in patient 1 (mmol/mol creatinine)

	During attack	*Fed*	*Fasted*	*MCT*
Adipate	380	16	21	245
Suberate	190	4	40	440
Suberylglycine	490	/	348	780
Sebacate	145	6	63	600
$C_6/C_8/C_{10}$ ratio	2.6/4.7/1.0			
3-Hydroxybutyrate	1367		41	2780
5-Hydroxyhexanoate	125		10	108
7-Hydroxyoctanoate	94		22	633

RESULTS

Liver histology. Needle liver biopsy examination revealed diffuse macrovesicular fatty changes, differing from the microvesicular changes reported in Reye syndrome.[5] Most of the liver cells contained a single large fat vacuole similar to those observed in carnitine palmityl transferase deficiency.[6] None of the mitochondrial abnormalities, as reported in Reye syndrome,[5] was observed on electron microscopy.

Organic acids. During the attack, patient 1 excreted abnormal amounts of fatty acid ω-oxidation products (i.e., adipate, suberate, and sebacate) as well as (ω-1)-oxidation products (5-hydroxyhexanoate and 7-hydroxyoctanoate, the corresponding ψ-hydroxy fatty acids[7]) (Table I). Plasma concentrations of adipate (29 μmol/L), suberate (27 μmol/L), and sebacate (33 μmol/L) were elevated well above normal values. The glycine conjugates of hexanoyl-CoA and suberyl-CoA were excreted in enhanced amounts. Among these abnormal metabolites, those derived from octanoate (i.e., suberate, suberylglycine, and 7-hydroxyoctanoate) predominated (Table I), indicating a maximal inhibition of the β-oxidation of C_8 mono- and dicarboxylic acids. Glutaric aciduria was within the normal range. During the days following the acute attack, none of the urine samples collected in the fed and fasting states contained significant amounts of the abnormal fatty acid oxidation products (Table I). Only MCT loading resulted in the recurrence of ethylmalonic, adipic, suberic, 7-hydroxyoctanoic, and sebacic acidurias.

3-Hydroxybutyric acid excretion was low during the attack and the 20-hour fast, and increased after MCT ingestion.

Neither patient 2 nor 3 excreted significant dicarboxylic or (ω-1)-hydroxy fatty acids when fasted. Because MCT ingestion was not well tolerated by patient 1, we did not use this test in his siblings or in other patients suspected of having a similar defect of fatty acid oxidation.

Carnitine and metabolic substrates during dietary studies. At the time of the attack in patient 1, free carnitine

Table II. Carnitine concentrations

	Plasma (μmol/L)		*Urine (μmol/gm creatinine)*		*Liver (mmol/mg protein)*
	Free carnitine	*Acylcarnitine*	*Free carnitine*	*Acylcarnitine*	*Total carnitine*
Patient 1					
Attack	47	15	74	1089	
Fasting			538	2423	3.0
Post-MCT			880	5760	
Patient 2	10	14	44	280	
Patient 3	66	29	38	185	
Controls (fasting)	79 ± 10	27 ± 8	81 ± 28	120 ± 39	13 ± 5

Table III. Medium-chain acyl-CoA dehydrogenase and glutamate dehydrogenase activities in disrupted fibroblasts

	Medium-chain acyl-CoA dehydrogenase (nmol/hr/mg)		*Glutamate dehydrogenase (μmol/hr/mg)*
	Substrate		
	Octanoyl-CoA	*Decanoyl-CoA*	
Patient 1	4.7	5.7	6.4
Patient 2	2.3	6.4	8.5
Patient 3	16.8 ± 2.5*	22.7	5.2 ± 0.2*
Controls (n = 4)			
Mean	29.0 ± 8.8*	26.8	5.2*
Range	24.1 to 53.9	24.8 to 30.5	3.3 to 8.3

*Mean ± SD, three different assays, each vs six controls.

levels were low in plasma, urine, and liver, whereas acylcarnitine excretion was increased (Table II). In the following days, with a normal diet without added carnitine, plasma and urinary free carnitine increased to the normal range, with a concomitant increment of acylcarnitines further amplified by orally administered MCT.

Plasma octanoate concentration (normal 6 to 13 μmol/L) was elevated in patient 1 at the time of the attack (53.4 μmol/L) and when fasted (22 μmol/L), as well as in patient 2 (20 μmol/L), but was normal in patient 3 (6 μmol/L).

During the fast, plasma glucose (60 and 63 mg/dl) and free fatty acid (2.7 and 2.4 mmol/L) concentrations were in the normal range in patients 1 and 2, respectively, whereas plasma concentrations of 3-hydroxybutyrate were inappropriately low (330 and 230 μmol/L) when compared with age-matched fasted controls.[8] The ingestion of MCT by Patient 1 did not change plasma glucose nor 3-hydroxybutyrate concentrations. Clinically it resulted in almost immediate depression of consciousness with alternating phases of obnubilation and wakefulness for the following 6 hours, similar to those reported in a 5-year-old child with multiple acyl-CoA dehydrogenase deficiency.[9]

Enzyme measurements. Studies of the two siblings' fibroblasts, collected respectively 3 months and 3 years after the episode of Reye-like syndrome, showed that the β-oxidation of fatty acids was defective at the level of MCADH (Table III). The lesser diminution of MCADH activity in the mother supported an autosomal recessive mode of inheritance.

DISCUSSION

The deficiency of MCADH was first demonstrated in fibroblasts[10,11] and hepatocytes[12] in children with clinical findings similar to those in our patients. This enzyme is one of five or more related intramitochondrial enzymes that dehydrogenate acyl-CoA esters at the alpha and beta carbons as the first step in the β-oxidation cycle. Absence of MCADH activity blocks the oxidation of medium-chain fatty acids, thereby preventing the oxidation of long-chain fatty acids beyond the first few turns of the β-oxidation cycle. Direct metabolic consequences of MCADH deficiency were observed in patient 1: suppression of fasting ketosis not restored by medium-chain fatty acid supply, and urinary excretion of organic acids reflecting the massive intrahepatic production of medium-chain acyl-CoA esters. The urinary pattern of organic acids observed has been reported in other children with MCADH deficiency,[11] and seems relatively specific for this disorder, inasmuch as it reflects a maximal inhibition of the β-oxidation of C_8 mono- and dicarboxylic acids.

Numerous reports of other recently identified genetic defects in fatty acid oxidation have shown that the manifestations of these disorders[9,13-18] and Reye syndrome[19] often develop in the days following a viral illness. Although the acute episodes in our patients both started after an upper respiratory tract infection, the children had several viral illnesses, including varicella, before and since the attack, without developing any detectable manifestations of a Reye-like syndrome. Infection,[20] and the accompanying nutritional deprivation and fasting because of vomiting, accelerate fatty acid mobilization. An excess supply of these substrates to a liver unable to oxidize them normally

could have contributed to the pathologic changes observed in patient 1, but none of the clinical or metabolic manifestations of the disease recurred during fasting, despite dramatic elevations of free fatty acid concentrations.

Inasmuch as the serum octanoate level remained normal during fasting, its elevation during the attack could not reflect the preexisting blockade of fatty acid oxidation at the MCADH level. After ingestion of octanoate in the form of MCT, the unequivocal manifestations of the disease in patient 1 suggest, rather, that the increase of plasma octanoate is one of the primary events triggering the onset of the Reye-like syndrome in patients with inherited MCADH deficiency.

Several reports have suggested the possibility that medications taken during the antecedent illness in patients with Reye syndrome may play a role in the development of the disease.[21] Inasmuch as both affected infants had been given salicylate during the days preceding the illness, the potential aggravating role of this drug was considered, but the two patients have ingested salicylate many times without any detectable clinical disorder.

The occurrence of a Reye-like syndrome in siblings should lead to suspicion of an inherited defect of fatty acid oxidation. Rather than prolonged fasting or MCT ingestion, which are of little help and potentially dangerous, investigation preferably should include gas chromatographic–mass spectrometric analysis of the urine at the time of the attack. If a characteristic organic acid profile, such as reported here, is documented, fatty acid oxidation should be studied in skin fibroblasts, because this technique can provide definitive identification of the MCADH genetic defect.

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