Short Communication

Significance of Bound Glutarate in the Diagnosis of Glutaric Aciduria Type I

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Glutaric aciduria type I (GA-I) (McKusick 231670) is an autosomal recessive disorder caused by glutaryl-CoA dehydrogenase (GD) deficiency. The diagnosis of GA-I is determined by organic acid analysis of urine and is confirmed by measuring the enzyme activity in cultured skin fibroblasts or leukocytes.

However, as new cases have been reported, they have revealed the difficulty of establishing the diagnosis by analysis of organic acids in urine alone, since glutaric acid excretion is not constant (Hellström 1982; Lipkin et al 1988; Bergman et al 1989; Haworth et al 1991). An alternative approach to the diagnosis is the determination of glutarylcarnitine in urine. Dorland et al (1990) described a permanent increase in conjugated urinary glutarate.

For these reasons, and according to our previous experience in a GA-I patient with repeated normal organic acid profile but slight increase in urine bound glutarate, we studied this parameter, together with plasma and urine carnitine and GD activity in three children with a clinical picture highly suggestive of GA-I.

MATERIAL AND METHODS

Normal controls: Thirty-four urine specimens from normal children were analysed. The ages of the infants ranged from 6 months to 10 years.

Patients: We studied urine, plasma, leukocytes or fibroblasts from four patients with classical GA-I; one patient with the intermittent form (urine was obtained during clinical stability); one GA-I patient with repeated normal urine organic acid profile; and three patients with bilateral striatal lesions in the basal ganglia (by CT and MRI) of unknown aetiology (Table 1) and normal organic acid profile.

Table 1 Clinical and biochemical findings of four patients with bilateral striatal lesions

Patient (sex)	Age at onset	Main clinical signs	Urinary glutarate (μmol/mmol creatinine)			Percent GD activity of parallel controls
			Total	Free	Bound	
D.M. (M)	7 m	Hypotonia; seizures; dystonia; tetraparesis; moderate megacephaly	20.0(crisis) 18.6 28.3 19.0	9.0 6.43 10.9 5.2	11.0 12.3 17.4 13.8	1 (fibroblasts)
Mc.M. (F)	23 m	Hypotonia; dystonia; tetraparesis	8.5	4.0	4.5	63 (leukocytes)
E.F. (M)	2 y 6 m	Altered consciousness; regression; dysarthria; pyramidal signs; asymmetric dystonia; tetraparesis	7.2	3.8	3.4	60 (leukocytes)
F.R. (M)	4 y 8 m	Fatiguability; hypotonia; asymmetric dystonia; tetraparesis	4.0	2.7	1.3	123 (leukocytes)
Controls (6 m–10 y) ($n = 34$; $\bar{x} \pm$ SD)			8.1 ± 4.0	5.7 ± 3.4	2.4 ± 2.0	
Classical GA-I ($n = 4$, range)			2814–8931	2105–7964	204–1839	0–3 (fibroblasts)
Intermittent GA-I on clinical stability ($n = 1$)			58.4	29.5	28.9	3 (fibroblasts)

Analytical procedure: Plasma free and total carnitine was determined by radioenzymatic assay (Barth et al 1983). GD dehydrogenase activity in fibroblasts or leukocytes was assayed as described previously (Christensen 1983). Urine and plasma glutarylcarnitine was determined by FAB–MS/MS.

Isolation of organic acids was done as described previously (Tanaka et al 1980) except that the urine samples were extracted three times with 2-ml portions of ethyl acetate and three times with 2-ml portions of diethyl ether. Trimethylsilyl derivatives were produced using BSTFA-pyridine and analysed by gas chromatography–mass spectrometry to confirm the identities of the compounds. To evaluate total glutarate, 1 ml of urine was subjected to mild alkaline hydrolysis by warming with 1 ml KOH (1 mol/L) at 38°C for 30 min, followed by acidification and isolation of organic acids as described above. Quantitation of free and total glutarate was done by gas chromatography using a fused silica capillary column (SPB-1, 30 m, 0.25 mm ID, 0.25 μm film thickness) and flame ionization detector (FID). Bound glutarate was calculated as the difference between total and free.

RESULTS AND DISCUSSION

The degree of organic aciduria in GA-I is very variable and it may even be absent. In these cases, measurement of glutarylcarnitine as well as plasma and urinary carnitine seems to be a valuable clue to the diagnosis and is used by some authors (Lipkin et al 1988; Bergman et al 1989; Hoffman et al 1991) when the urinary organic acid profile is non-informative.

Measurement of urinary glutarylcarnitine by fast-atom bombardment tandem mass spectrometry (FAB–MS/MS) is not available in most laboratories. For this reason we established normal glutarate levels before (free glutarate) and after a mild alkaline hydrolysis (total glutarate), in order to evaluate its increase (bound glutarate), which is mainly due to glutarylcarnitine in patients with GA-I. Small values of bound glutarate were found in normal controls (Table 1).

As expected, classical GA-I patients showed high, unambiguous values. Although not so high, the significant increase in bound glutarate can help the diagnosis of GA-I in patients with slight increase of free glutarate, such as those with the intermittent form (Table 1), especially when the urine sample is collected during clinical stability.

Patient D.M. with 1% residual GD activity, poses more difficulties in the diagnosis of GA-I, because free glutarate, as well as the whole urine organic acid profile, was always normal, even during episodes of clinical decompensation. Total glutarate (in four independent samples) was always in the control range, but it was about twice the amount of free. The slight but significant increase of bound glutarate, over 3SD above the control mean, was well correlated with a significant increase of glutarylcarnitine in urine determined by FAB–MS/MS.

The other three patients included in this study, with a clinical picture strongly suggestive of GA-I, failed to demonstrate any significant increase of bound glutarate and GD determination resulted in normal activities.

Haworth et al (1991) did not find any increase of bound glutarate in their patients, but it appeared to be permanent in ours, in agreement with Dorland et al (1990).

Our method has sufficient sensitivity to quantitate normal values of glutarate (limit of sensitivity of FID is about 0.1 μmol/mmol creatinine). In fact, determination of free glutarate in D.M. by isotopic dilution GC–MS gave similar normal results (5.0 and 3.7 μmol/mmol creatinine) in two different samples. Nevertheless, the authenticity of any significant increase in total glutarate must be verified by GC–MS, in order to avoid false positive results. Stronger conditions of temperature, basicity and time of incubation (100°C, 5 mol/L KOH, 1 h) produced the same glutarate increase.

The pattern for plasma and urine carnitine was clearly altered in classic and intermittent GA-I patients, with low free- and high acyl-carnitine. In contrast, total-, free- and acyl-carnitine levels were completely normal in plasma and urine of patient D.M., as well as in the other three GD non-deficient patients.

We conclude that evaluation of bound glutarate in urine is a useful aid in some difficult diagnoses of GD deficiency, and the analytical procedure is available for most laboratories.

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