J. Inher. Metab. Dis. 16 (1993) 753–761

L-2-Hydroxyglutaric Acidaemia: Clinical and Biochemical Findings in 12 Patients and Preliminary Report on L-2-Hydroxyacid Dehydrogenase

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Summary: L-2-Hydroxyglutaric acidaemia represents a newly defined inborn error of metabolism, with increased levels of L-2-hydroxyglutaric acid in urine, plasma and cerebrospinal fluid. The concentration in cerebrospinal fluid is higher than in plasma. The other consistent biochemical finding is an increase of lysine in blood and cerebrospinal fluid, but lysine loading does not increase L-2-hydroxyglutaric acid concentration in plasma. This autosomal recessively inherited disease is expressed as progressive ataxia, mental deficiency with subcortical leukoencephalopathy and cerebellar atrophy on magnetic resonance imaging. Since these features were described in 8 patients by Barth and co-workers in 1992, 4 more patients with similar findings have been diagnosed and added to the present series. L-2-Hydroxyglutaric acid is found in only trace amounts on routine gas chromatographic screening in normal persons, and its origin, its fate and even its relevance to normal metabolism are unknown. Therefore its catabolism was studied in normal liver. Incubation of rat liver with L-2-hydroxyglutaric acid did not produce H_2O_2, which excluded (peroxisomal) L-2-hydroxyacid oxidase as the main route of catabolism. However, L-2-hydroxyglutaric acid is rapidly dehydrogenated if NAD^+ is added as a co-factor to the standard reaction medium. This could also be demonstrated

in human liver. The preliminary evidence for this enzyme activity in rats and humans, L-2-hydroxyglutaric acid dehydrogenase, is given. Further investigations are required to clarify the possible relevance to the metabolic defect in L-2-hydroxyglutaric acidaemia.

Since the description of the first case of L-2-hydroxyglutaric aciduria (Duran et al 1980) in a 5-year-old boy with psychomotor retardation and dystrophy, an abnormality of white matter suggestive of leukodystrophy has been reported in other patients (Jaeken et al 1988; Hoffmann et al 1990). A recent report (Barth et al 1992) was presented based on 8 patients from various European countries, including the patients previously reported. Autosomal recessive inheritance was strongly suggested by the family histories. Similar neurological abnormalities were present in each case, with a progressive course of mental deficiency, ataxia and other motor system deficiencies. Increased L-2-hydroxyglutaric acid was found in urine, plasma and CSF of all patients investigated. Computerized tomography (CT), and especially magnetic resonance imaging (MRI) showed a consistent pattern of subcortical leukoencephalopathy. Furthermore, cerebellar atrophy and signal changes in several nuclei were found, together representing a pattern not found in other known neurodegenerative disorders.

In this communication four more patients are included in the series, giving added evidence for the genetic origin, the specific pattern of neurological affection involved and the accompanying abnormalities on MRI. Comparison is made to L-glutaric aciduria type I (McKusick 23167).

In order to find a metabolic role for L-2-hydroxyglutaric acid, its metabolic fate in homogenates of normal rat and human liver was studied by enzymatic methods and the findings – a newly discovered type of L-2-hydroxyglutaric acid dehydrogenase activity – are reported.

METHODS

Organic acids in body fluids were assayed by GC–MS. Identification of L-2-hydroxyglutaric acid and determination of its absolute configuration as the L-stereoisomer were performed as previously described (Duran et al 1980). Amino acids were quantified by automatic amino acid analysis. Pipecolic acid was determined as previously described (Kok et al 1987).

Fibroblasts from patients and controls were incubated with 12 μCi [1-^{14}C]-2-oxoglutaric acid for 48 h. Thereafter the medium and the cells were combined, organic acids were separated by liquid partition chromatography as previously described (Sweetman 1974) and the radioactivity in 2-hydroxyglutaric acid was determined. The amount of 2-oxoglutaric acid metabolized via the citric acid cycle was estimated by determining the production of $^{14}CO_2$.

The activities of L-2-hydroxyglutaric acid oxidase and L-2-hydroxyglutaric acid dehydrogenase in homogenates of rat liver as well as human livers were measured according to the following procedures. For L-2-hydroxyglutaric acid oxidase activity measurements, the reaction medium contained the following standard components: 100 mmol/L Tris-HCl (pH 8.8), 0.05% (w/v) Triton X-100, 6 mmol/L 2,4,6-tribromohydroxybenzoate and 1 mmol/L aminoantipyrine. Reactions were initiated by adding

10 mmol/L L-2-hydroxyglutarate (sodium salt) and the absorbance was measured at 510 nm as previously described (Wanders et al 1989). For L-2-hydroxyglutarate dehydrogenase activity measurements, the same reaction medium was used except that 5 mmol/L NAD^+ was added. Reactions were started by adding 10 mmol/L L-hydroxyglutarate and the absorbance at 340 nm was followed using a COBAS-BIO centrifugal analyser (Hoffman-LaRoche, Basle, Switzerland).

PATIENTS

Clinical findings: These are summarized in Table 1. As a rule no abnormalities were noted in the first year of life. Thereafter, delay in unsupported walking (A1, B1, D1, E2), abnormal gait (C1), speech delay (A2), or severe febrile seizures (D5) were the presenting symptoms in early childhood in 7 patients. In 4 patients no abnormalities were noted until learning disabilities in the first school years drew attention to their handicap. In one patient (E1) cerebellar signs at age 10 years were the first symptom. Seizures, either febrile or grand mal or both, were present in 6 patients. The main motor handicap was cerebellar dysfunction (dysarthria, truncal ataxia, dysmetria and gait ataxia are reported individually and in variable combinations). Cerebellar symptoms were indeed the most common abnormality, after mental deficiency. These were present in all patients over 15 years of age.

There are strong indications for autosomal recessive inheritance, since 4 of 7 families had more than one affected child, and in two families the parents were consanguineous (D and G). Males and females are similarly affected. The ratio between affected and unaffected sibs is 12/12. Further reporting will enlarge the series and may give the true proportions between males and females, as well as the proportion between affected and unaffected sibs. The finding that 4 of the 7 families had more than one affected child may in part be explained by increased demand for expert diagnostic help in the case of families with more than one affected child, and

Table 1 Clinical profiles of affected patients

Patient no.[a]	*Sex*	*Age*[b] (years)	*Mental deficiency*	*Cerebellar symptoms*	*Extrapyramidal symptoms*	*Pyramidal symptoms*
A1	M	19	+	+	+	−
A2	M	13	+	−	−	−
B1	F	20	+	+	+	−
C1	F	16	+	+	−	+
D1	F	19	+	+	−	−
D2	F	15	+	+	+	−
E1	M	39	+	+	+	+
E2	M	33	+	+	−	−
F1	F	16	+	+	−	−
F2	F	16	+	+	−	−
F3	F	11	+	+	−	−
G	F	12	+	+	−	−

[a]Affected sibs are indicated by same letter
[b]Age at last follow-up

this in turn may explain the 1/1 ratio of affected/unaffected sibs, rather than the 1/4 expected on the basis of autosomal recessive inheritance. The ethnic origins of the affected families were in different regions: Morocco, Turkey, Greece, Germany, Italy and Latin America.

Neuroimaging studies: The results of neuroimaging have been published previously for the patients A1 through E2 (Barth et al 1992). Briefly, the most specific abnormality was loss of subcortical white matter. This was demonstrable by CT, but could be demonstrated more convincingly by MRI, where loss of signal in T_1-weighted sections and increased signal on T_2-weighted sections was found in the subcortical regions. The internal and external capsules were moderately involved. Periventricular white matter was much less or not involved. The caudate nuclei were atrophic; the putamen showed signal changes; the pallidum, thalamic nuclei, mesencephalon and lower brainstem seemed uninvolved. The cerebellum was affected by folial atrophy, mainly affecting the vermis, and signal changes in the dentate nuclei. Two new MRI series not previously described were added to this patient series (patients F1 and G1). Review of their MRI disclosed the same pattern as previously described, except that in the case of patient G1 there was more extensive involvement of the deep frontal white matter in addition to the typical findings described above. A typical example is given in Figure 1.

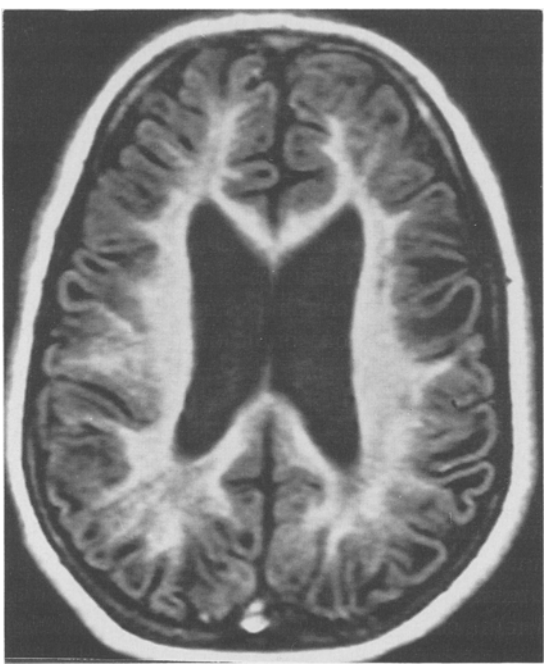

Figure 1 T_1-weighted axial MR section from patient A1. Subcortical white matter is severely deficient, shown by the nearly empty appearance of the gyral cores. There is moderate signal loss in the central white matter

RESULTS

Results of determinations of L-2-hydroxyglutaric acid in plasma, CSF and urine, and of lysine in plasma and CSF are given in Table 2. Elevations were found in each patient. The results of paired samples from CSF and plasma showed that the concentration of L-2-hydroxyglutaric acid in the CSF was higher than in plasma in all cases where paired samples were available ($n = 6$). This is represented in Figure 2, where paired plasma and CSF concentrations are plotted against each other. Lysine was elevated in all samples of plasma and CSF examined. Plotting of the concentrations of plasma and CSF against each other shows that plasma levels were higher than CSF except in one patient (Figure 3).

Table 2 Concentration of metabolites in body fluid

	Patients		*Controls*	
Body fluid	*2OHglu ac.*[a]	*Lysine*	*2OHglu ac.*[a]	*Lysine*
Plasma (μmol/L)				
Range	7–84	70–380	ND[c]	120–230
Mean[b]	31 (10)	279 (8)		
CSF (μmol/L)				
Range	23–474	66–89	ND	10–25
Mean	122 (6)	79 (6)		
Urine (mmol/g creatinine)				
Range	2–38	0.10–0.37	< 0.46	0.05–0.40
Mean	16 (12)	0.24 (4)		

[a]20Hglu ac. = L-2-hydroxyglutaric acid
[b]Number of patients in parentheses
[c]ND = not detectable

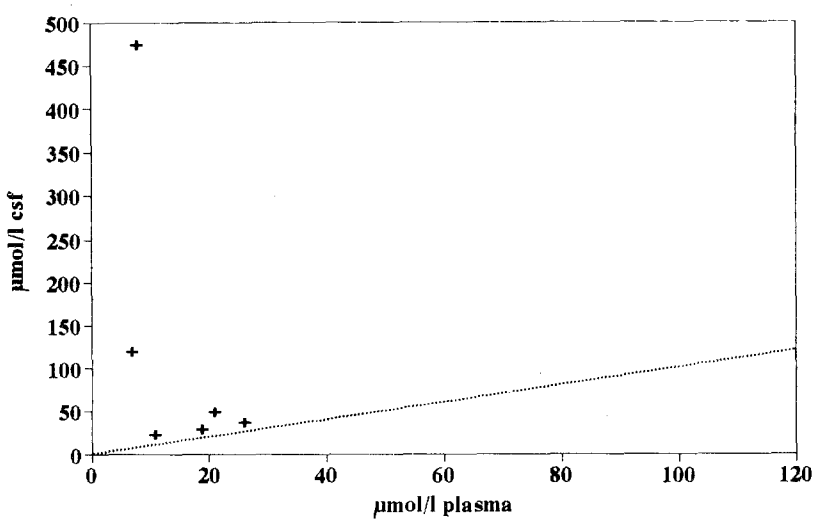

Figure 2 L-2-Hydroxyglutaric acid concentrations in plasma and CSF samples drawn at the same time plotted against each other

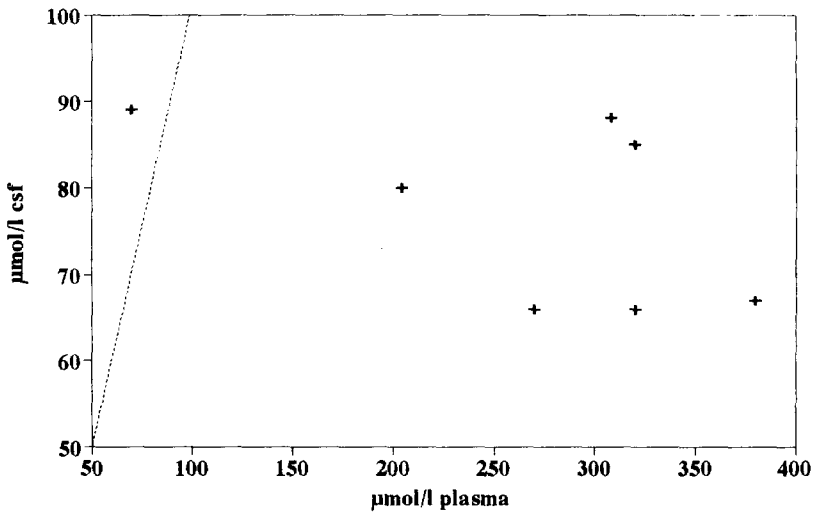

Figure 3 Lysine concentrations in plasma and CSF samples drawn at the same time plotted against each other

Pipecolic acid was determined in plasma and in CSF from patients E1 and E2 with normal results. No excesses of L-2-hydroxyacids other than L-2-hydroxyglutaric acid were found by the procedures employed. 2-Oxoglutaric acid was not elevated in any of the samples of plasma or CSF investigated. Long-chain and very long-chain fatty acids (C_{16} to C_{26}) were examined by GC–MS in patients A1 and A2, with normal results. Furthermore, miscellaneous investigations with normal results in at least one patient each included *N*-acetylaspartic acid excretion, purine and pyrimidine screening by two-dimensional thin-layer chromatography in urine, and phytanic acid in plasma. The amount of $^{14}CO_2$ recovered by the incubation of fibroblasts with [1-^{14}C]-2-oxoglutaric acid was comparable between cell lines of controls and patients. In addition, a significant peak of radioactivity was recovered in 2-hydroxyglutaric acid. It was identical in patients and controls.

No H_2O_2 was produced on incubation of human and rat liver with L-2-hydroxyglutaric acid. The results of L-2-hydroxyglutaric acid dehydrogenase activity in normal human and rat liver are given in Table 3.

Table 3 L-2-Hydroxyglutaric acid dehydrogenase activity in liver: summary of its properties

	Rat	*Human*
pH optimum	8.7	ND[a]
K_m (NAD^+)	0.4–1.0 (3)	ND
K_m (2OH-glu ac.)[b]	5.0–10.0 (3)	ND
Activity in liver (nmol/min per mg)	35.1 ± 0.9 (5)	28.2 ± 5.1 (4)

[a]ND = not determined
[b]2OHglu ac. = L-2-hydroxyglutaric acid

DISCUSSION

The disease pattern described above is fairly specific from the viewpoint of neurological and MRI findings. It should be pointed out that no periods of metabolic acidosis and no acute deteriorations were found in any of the patients, but rather a chronic, slowly progressive disorder with insidious onset after infancy. A related compound, glutaric acid, is specifically elevated in glutaric acidaemia type I, which results from a defect of glutaryl-CoA dehydrogenase (EC 1.3.99.7) in the degradation of lysine and tryptophan.

The history of patients with glutaric acidaemia type I is very different from that of patients with L-2-hydroxyglutaric acidaemia. In the former, the usual picture is one of early-onset dystonic cerebral palsy, in most cases with an acute onset in infancy (Haworth et al 1991; Hoffmann et al 1991). Diminished serum free L-carnitine and intermittent dicarboxylic aciduria point to secondary involvement of mitochondrial fatty acid metabolism. In the case of L-2-hydroxyglutaric acidaemia no acute encephalopathic episodes, no abnormalities of carnitine metabolism or excess of dicarboxylic acids have been found in any of the patients investigated. L-2-Hydroxyglutaric acid is higher in CSF than in plasma in all 7 cases thus far investigated. This suggests an endogenous origin of the compound, although a bacterial origin has been described (Reeves and Ajl 1962). The increased CSF-to-plasma ratio may be due to a specific role of L-2-hydroxyglutaric acid in cerebral metabolism.

The stereotyped MRI pattern is a subcortical encephalopathy, whereas myelin loss is mainly periventricular in all the leukodystrophies known. For the pattern of demyelination, comparison may be drawn with Canavan's spongiform encephalopathy (aspartoacylase deficiency), where myelin breakdown also is predominantly subcortical rather than periventricular. Together with the other findings, especially the cerebellar atrophy and the signal changes in several nuclei that are important in the organization of voluntary movement, such as the putamen and the caudate and dentate nuclei, the pattern is highly specific. Therefore, it should easily be recognized by neuroradiologists while investigating children or adults for mental deficiency and progressive ataxia. This finding should prompt GC–MS investigation of urine, plasma and CSF, when this has not been done previously.

So far there is no definite clue to the metabolic block involved. A number of loading and fasting studies have been conducted without conclusive results (Barth et al 1992). The consistent elevation of lysine in the patients drew our attention to lysine catabolism. We previously reported that L-lysine loading (75 mg/kg body weight) in two of the patients did not result in any change of the basal plasma level of L-2-hydroxyglutaric acid, even though the absorption of lysine was quite efficient (Barth et al 1992). Since the principal catabolic pathway for L-lysine in the brain has been reported to be the pipecolic acid pathway, rather than the saccharopine pathway (Chang 1976) the levels of pipecolic acid in CSF and in plasma were determined in two patients. The results were normal. Therefore, there is little evidence for a primary block in lysine catabolism to explain the findings. Earlier, Jaeken and colleagues (1988) proposed that the raised lysine could originate from a possible interference of an excess of L-2-hydroxyglutaric acid with lysine breakdown through competitive

inhibition, possibly with 2-oxoglutarate at the level of saccharopine dehydrogenase.

Two approaches were tried to gain information on the metabolic pathways related to L-2-hydroxyglutaric acid. First, we tried to obtain information on the formation of 2-hydroxyglutaric acid by studying the turnover of radiolabelled 2-oxoglutaric acid in fibroblasts of patients and controls. By using [1-^{14}C]-2-oxoglutaric acid, labelled at the first carbon, the label of the compound should have been lost if it was metabolized via the citric acid cycle. The amount of 2-oxoglutaric acid metabolized via the citric acid cycle was estimated by determining the production of $^{14}CO_2$ and was comparable between cell lines of controls and patients. In addition a significant peak of radioactivity was recovered in 2-hydroxyglutaric acid. It was identical in patients and controls. This implied that 2-hydroxyglutaric acid was formed from 2-oxoglutaric acid in human fibroblasts. However, there was no apparent difference between patients and controls. As it was not possible to differentiate between the formation of the two isomers of D- and L-2-hydroxyglutaric acid by this approach, it cannot be excluded that the assay tested the formation of D-2-hydroxyglutaric acid and not L-2-hydroxyglutaric acid.

We then tested the metabolic fate of L-2-hydroxyglutaric acid in tissues. First, we investigated the possibility that L-2-hydroxyglutaric acid reacts with an oxidase, which uses molecular oxygen as a substrate, subsequently yielding hydrogen peroxide (H_2O_2). In the past several L-2-hydroxyacid oxidases have been identified, including L-2-hydroxyacid oxidase A and B in liver and kidney, respectively, from rat and other species (Nakano et al 1968; Yokota et al 1985). These experiments, in which the homovanillic acid/peroxidase system was used to measure the production of hydrogen peroxide (Wanders et al 1989), revealed that L-2-hydroxyglutaric acid is not degraded in an oxidase type reaction, which excluded a significant role for peroxisomal L-2-hydroxyacid oxidases. Subsequently, we investigated whether L-2-hydroxyglutaric acid is degraded in a dehydrogenase type reaction. These studies showed that L-2-hydroxyglutaric acid is indeed rapidly dehydrogenated if NAD^+ is added as a cofactor to the standard reaction medium. The properties of this enzyme activity as listed in Table 3 show that the enzyme as present in rat liver has a relatively high pH-optimum (8.7) and a low affinity for L-2-hydroxyglutaric acid. Importantly, the enzyme activity was found to be about equal in human liver specimens. In the past the occurrence of L-2-hydroxyglutaric acid as an intermediate in rat tissues was reported by Weil-Malherbe (1937), but the significance of this metabolite for man was not raised before the discovery of L-2-hydroxyglutaric acidaemia. Subsequent studies in patients will be required to reveal whether the accumulation of L-2-hydroxyglutaric acid is indeed due to a deficiency of this newly identified enzyme activity. Such studies are underway.

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