Biochemical Contribution to Diagnosis and Study of a New Case of D-Glyceric Acidemia/Aciduria

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During organic acid screening by gas chromatography/mass spectrometry, we detected a large peak corresponding to glyceric acid in a patient's urine sample. The D(+)-configuration was demonstrated by a polarimetric method and by enzymatic stereospecificity of D-glycerate dehydrogenase (EC 1.1.1.29). We biochemically investigated this fifth reported case of d-glyceric acidemia. In our patient, loading tests with L-serine and fructose led to an increase of D-glyceric acid in both plasma and urine. Determination of other metabolites involved in D-glycerate metabolism revealed no abnormality in any sample examined. After comparing all our results with those of the preceding observations described in the literature, we suggest a possible enzymatic defect located on one of the metabolic pathways shared by fructose and L-serine, possibly at the level of hepatic D-glycerate kinase (EC 2.7.1.31). Nevertheless, a primary defect of L-serine catabolism cannot be entirely excluded.

Additional Keyphrases: metabolism · heritable disorders · gas chromatography/mass spectrometry · organic acids · urine · D-glycerate dehydrogenase

Urinary excretion of glyceric acid is particularly rare and is found in only two metabolic disorders: D-glyceric and L-glyceric acidurias. The two can be distinguished according to clinical picture and urinary organic acid profile. In D-glyceric aciduria, only glyceric acid appears as the pathological metabolite, whereas in L-glyceric aciduria (primary hyperoxaluria type 2), increased amounts of glyceric acid are associated with another abnormality such as increased oxalic acid, which causes clinical symptoms of recurrent nephrolithiasis (1-3).

This paper reports a new case of d-glyceric acidemia/aciduria, studied essentially by gas chromatography and mass spectrometry (GC/MS).6 To date, excretion of abnormally high amounts of D-glyceric acid has been observed in only four cases, reported from 1974 to 1987 (4-8).

Here we report a fifth case of this metabolic disease. On the basis of biochemical investigations, we compare it with the four observations previously described in the literature and attempt to elucidate the metabolic lesion.

Case Report

Patient C. E., a nine-month-old girl, is the first child of consanguineous Turkish parents. During her admission in the pediatric clinic, she presented with delayed psychomotor development, axial hypotonia, and spastic tetraparesis. Results of routine biochemical investigations were normal; in particular, no metabolic acidosis was detected. Study of amino acids in serum and urine showed no notable abnormality.

However, during screening of urinary organic acids by GC/MS, large amounts of an unusual compound, glyceric acid, were found repeatedly. The patient was on a normal diet and no treatment was required. Organic acid profiles showed no artefact attributable to drugs or dietary constituents.

Materials and Methods

Samples. We collected 24-h urine specimens in four 6-h portions, without any additive. Plasma (obtained by use of heparinized tubes), cerebrospinal fluid, and urine specimens were stored at −20 °C until extraction.

Instrumentation. We used a gas chromatograph (Model 8500; Perkin-Elmer, Bucks., U.K.) equipped with flame ionization detector and connected with a Perkin-Elmer GP 100 graphic printer. A 25 m × 0.22 mm (i.d.) WCOT fused-silica capillary column CP SIL 5CB (Chrompack, Middelburg, The Netherlands) was selected. The trimethylsilyl (TMS) derivatives of organic acids were identified by combined GC/MS with use of a Hewlett-Packard 5993-B spectrometer (Hewlett-Packard, Palo Alto, CA) operated with no. 5985 software. The same capillary column was connected to the ion-source of the mass spectrometer with a platinum restrictor, which splits the flow at the entry of the ion-source.

Reagents and standards. All solvents were "HPLC" grade. Bis(trimethylsilyl)trifluoroacetamide/trimethylchlorosilane (99/1 by vol) was from Pierce Chemical Co., Rockford, IL. Methoxyamine hydrochloride and D-glycerate dehydrogenase (from spinach leaves, EC 1.1.1.29) were from Sigma Chemical Co., St. Louis, MO.

D(+)- and L(-)-glyceric acid (hemi-calcium salt), D(+)-glyceraldehyde, n-alkanes (from C10 to C24), and β-hydroxy- pyruvic acid were from Sigma Chemical Co.; 2-phenylbutyric acid, used as internal standard, was from Aldrich Chemie, Steinheim, F.R.G.

Extraction and derivatization. To achieve reproducible
recovery of about 25% (CV 4.7%) we standardized the organic acid extraction as follows. Add one volume of sodium hydrochloride (saturated solution) and 0.1 volume of internal standard (0.15 g/L solution) to the sample. After acidification with concentrated hydrochloric acid, extract (five times) the organic acids by automated shaking with four volumes of ethyl acetate during 2 min. After dehydration over sodium sulfate, evaporate the combined extracts under nitrogen at 40 °C, during about 15 min. Dissolve the residue in 3 mL of acetone and then evaporate under the same conditions. Add 100 μL of bis(trimethylsilyl)trifluoroacetamide/trimethylchlorosilane and 250 μL of chloroform to the dry mixture, and derivatize at 80 °C for 20 min. Add the n-alkanes mixture (12.5 g/L of each) before injection onto GC column.

A preliminary deproteinization with nine volumes of ethanol was necessary for plasma samples. We analyzed D-glyceraldehyde and β-hydroxypyruvic acid as TMS-methoxime derivatives (9).

Gas chromatography procedure. The analytical procedure was as follows: carrier gas helium at a pressure of 83 kPa; injector temperature 300 °C; detector temperature 300 °C; initial oven temperature 65 °C for 6 min, then programmed to 250 °C at a rate of 4 °C/min, with the final temperature maintained for 5 min. Mass spectra were recorded at an ionization potential of 70 eV. Identification of all TMS derivatives, according to their retention times and methane unit values, was confirmed by GC/MS.

Quantitative analysis of glyceric acid. We determined concentrations of this metabolite by two different methods: GC, with reference to calibration curves established with D-glyceric acid standards solubilized in the same biological fluids as the analyzed samples, and a fully automated procedure on a centrifugal analyzer (Cobas Bio, Roche S. A., Neuilly, France), based on use of D-glycerate dehydrogenase, which is specific for the D-form of glyceric acid (10). Urinary glyceric acid concentrations were expressed in ratios with creatinine excretion, creatinine being usually considered as the best reference in quantitative studies of organic acidurias, particularly in the newborn (11).

Characterization of glyceric acid stereoisomer form. Several methods can be used for the determination of the enantiomer form of glyceric acid: GC analysis of O-acetylated methyl esters (12), polarimetry (10, 13), and enzymatic stereospecificity of D-glycerate dehydrogenase (10, 13). We performed these two last methods in our laboratory. We isolated glyceric acid from the patient’s urine by anion-exchange chromatography on diethylaminoethyl-Sephadex A-25, according to Chalmers and Watts (14). We studied optical rotatory dispersion between 286 and 365 nm by using a Perkin-Elmer 141 M.C. spectropolarimeter. The configuration was also confirmed by the enzymatic specificity of D-glycerate dehydrogenase—both by measuring variation of optical rotatory dispersion and by detecting at 340 nm the NADH generated.

Quantitative analysis of other metabolites. We determined oxalic acid and glycolic acid in urine by GC. In plasma and urine, we evaluated glycerol by a classical enzymatic procedure, using a test combination from Boehringer, Mannheim, F.R.G. All the metabolites in the 2-phospho-D-glycerate pathway were simultaneously studied with reagents from the 2,3-diphosphoglycerate test combination (Boehringer).

Results

Urinary organic acid screening and glyceric acid characterization. All the chromatograms showed a large peak (methylen unit value, 13.49) that was identified as glyceric acid by mass spectrometry. No other abnormal peaks were detected, but to rule out the possibility of an L-glyceric aciduria, we checked for oxalic acid in a few urine specimens. Oxalic acid concentrations ranged from 0 to 0.35 mmol/L [normal range 0.11–0.50 mmol/L (7)]. Glycerolic acid was also in the normal range, varying from 60 to 102 μg/mg creatinine [normal value in children ages 1–3 years, from Thompson et al. (15): 112 ± 44 μg/mg creatinine]. Therefore, interference from this metabolite was unlikely during enzymatic assay with D-glycerate dehydrogenase (16).

Stereosomer form of glyceric acid. We obtained a positive optical rotatory dispersion with all urinary extracts and observed a progressive decrease of the values during incubation with D-glycerate dehydrogenase. These results confirmed that the configuration of glyceric acid was D. Because similar amounts were obtained by both the GC method and the enzymatic method, we concluded that the patient’s glyceric acid was exclusively in the D(+) configuration.

Variations of glyceric acid amounts. Concentrations of glyceric acid varied little during a day and from day to day: in urine they ranged from 32.3 to 57.6 mmol per gram of creatinine and in plasma from 29.8 to 62.2 mg/L (281–587 μmol/L). In the patient’s cerebrospinal fluid, glyceric acid was significantly higher than the trace amounts found among healthy children.

Loading tests. To determine whether the metabolic lesion was affecting the fructose or the L-serine pathway, we performed oral loading tests with fructose (1 g/kg of body weight) and with L-serine (200 mg/kg), then monitored the subsequent plasma concentrations of fructose, glucose, lactate, L-serine, L-glycine, and D-glyceric acid as well as the concentrations of D-glyceric acid in urine. We interpreted the loading tests essentially by considering the variations in the concentrations in plasma: during the L-serine loading test, plasma L-glycine remained constant, but a peak of L-serine, recorded 45 min after the L-serine loading, progressively decreased by about 600 μmol/L between 45 and 180 min. During the same time interval, glyceric acid increased by about 500 μmol/L in plasma (Figure 1). Urinary glyceric acid showed a significant increase of about threefold the usual excretion rate: from 44.4 mmol/g creatinine before L-serine ingestion to 120 mmol/g creatinine in the first 6 h afterwards. We concluded that a part of L-serine ingested was converted into D-glyceric acid.

Fig. 1. Variations of D-glyceric concentrations in plasma (left) and in urine (right) during oral loading tests: A, fructose loading; B, L-serine loading.
On the other hand, during the fructose loading test, we observed the following: in plasma, fructose increased rapidly, by 233 μmol/L after 15 min, then decreased while glyceric acid was increasing by 120 μmol/L (Figure 1). In urine, glyceric acid increased from 64 to 83.7 μmol/g creatinine. Consequently, in our patient, both fructose and L-serine loading tests led to an increase of glyceric acid, but the most impressive response was during the L-serine test. D-Glyceric acid remained undetectable in urine specimens from the patient’s parents before and after their fructose loading test. We could not test them with L-serine loading.

Dietary restriction tests. We placed the patient on a fructose-free diet and a protein-restriction diet for several days. We saw no decrease of D-glyceric acid excretion after those restriction tests.

Study of other intermediate metabolites. All the metabolites involved in the different metabolic pathways of glyceric acid were investigated during the loading tests (see Figure 2). D-Glyceraldehyde and β-hydroxybutyric acid remained undetectable even in fresh plasma urinary specimens. We obtained the same results for plasma samples.

Free glycerol concentrations in plasma and urine showed no abnormality: from 5.6 to 14.6 mg/L in plasma (normal range: 5–17 mg/L) and from 0 to 12 mg/L in urine without any variation during loading tests. Determination of all the metabolites of the 2-phospho-D-glycerate metabolic pathway (2-phospho-D-glycerate, 3-phospho-D-glycerate, 3-phospho-D-glyceraldehyde, and dihydroxyacetone phosphate) revealed no increase in any of them in any sample examined.

Discussion

From the results given here, it becomes clear that our patient suffers from D-glyceric acidemia. Table 1 summarizes the heterogeneity in the cases of D-glyceric acidemia described in the literature since 1974. Except for the patient of Wadman et al. (6), who exhibited mainly a severe metabolic acidosis, the clinical picture described consists of progressive encephalopathy.

Among routine biological results, hyperglycinemia was observed by Brandt et al. (4, 5) and by Grandgeorge et al. (7) in a lethal acute form of D-glyceric acidemia. Wadman et al. (6) reported a metabolic acidosis. In the present case, as in the observations of Duran et al. (8), no typical abnormality appeared: only the study of urinary organic acids led to the diagnosis of glyceric acidemia. Glyceric acid is rarely found in human urine, as demonstrated by Kölvsaa et al. (10). However, Chalmers et al. (17) detected traces of glyceric acid in urine from a patient with propionic acidemia. In our experience, we have observed this metabolite only in a child suffering from neonatal adrenoleukodystrophy. D-Glyceric acid excretion in our patient appeared relatively important, with moderate variations during the day and from day to day. The patient of

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**Table 1. Comparison of Our Observations with the Four Cases of Glyceric Acidemia Described in the Literature**

<table>
<thead>
<tr>
<th>Observations</th>
<th>Brandt et al., 1974</th>
<th>Wadman et al., 1975</th>
<th>Grandgeorge et al., 1980</th>
<th>Duran et al., 1987</th>
<th>Our patient</th>
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<tbody>
<tr>
<td>Metabolic acidosis</td>
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<td>+ + +</td>
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<td>Hyperglycinemia</td>
<td>+ + +</td>
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<tr>
<td>D-Glyceric acid</td>
<td>In urine, mmol/L</td>
<td>14.1–23.6</td>
<td>13.9–116</td>
<td>1.98–15.6</td>
<td>0.56–9.3</td>
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<td></td>
<td>(mmol/g creatinine)</td>
<td>(93.9–286)</td>
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<td>In plasma, μmol/L</td>
<td>1000–1300</td>
<td>&lt;4000</td>
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<td>Loading tests</td>
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<td>Dihydroxyacetone</td>
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<td>L-Serine</td>
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<td>Also detected in urine</td>
<td>Hydroxybutyric acid</td>
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<td>D-Glyceraldehyde</td>
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<td>Metabolic lesion hypotheses</td>
<td>D-Glyceraldehyde</td>
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<td>and possible enzymatic defect</td>
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<td>D-Glyceraldehyde</td>
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<td>Glycerol dehydrogenase</td>
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*In 1980 an alternative proposal involved inhibition of glycine catabolism by acylcoenzymes A (22).
Wadman et al. (6) showed a still greater excretion, whereas that of Duran et al. (8) exhibited a moderate excretion of glyceric acid. Both those cases displayed considerable variation of excretion. In our patient, measurements of D-glyceric acid concentrations in plasma were constantly greater than in the patient reported by Duran et al., but lower than in the patients of Brandt et al. and Wadman et al.

Because D-glycerate is produced at the junction of the metabolic pathways of L-serine and fructose, we performed restriction and loading tests to understand its accumulation. Our patient reacted both to L-serine and fructose loading tests, with a better response to L-serine, whereas in the patient of Wadman et al., only L-serine had a positive effect on D-glycerate excretion. On the contrary, Duran et al. obtained an impressive response to fructose and dihydroxyacetone. In the patient of Brandt, only the oral fructose loading test was performed (reported by Kevilraa et al. (18)), which led to an increase of D-glycerate excretion. In our case, it is evident that both L-serine and fructose can be thought to be precursors of D-glycerate biosynthesis. Our observation differs from the other cases because our patient is the only one who reacted to the two loading tests.

Three metabolic pathways are involved in human D-glycerate metabolism (see Figure 2). In our patient, the loading tests give us the following information. D-Glycerate dehydrogenase integrity was proved by the metabolic effects of L-serine: increasing D-glycerate concentrations and lack of accumulation of hydroxypropane and l-glycerate during the test. Aldehyde dehydrogenase deficiency is unlikely because our patient also reacted to the fructose loading test with increases in D-glycerate. During this test, D-glycerate excretion was less impressive than in the patient of Duran et al. Therefore, we conclude that, unlike their patient, ours does not seem to be defective in triokinase, the major enzyme involved in D-glyceraldehyde metabolism (19, 20).

Thus, as judged by the results of the two loading tests, the primary enzymatic defect must be located on a metabolic pathway shared by fructose and L-serine. Consequently, only a deficiency of D-glycerate kinase (EC 2.7.1.31) would explain such a D-glycerate accumulation. Nevertheless, considering the low activity of D-glycerate kinase in human liver (21), a metabolic lesion located on one of the major L-serine catalytic pathways cannot be totally ruled out. In this case, D-glycerate biosynthesis from L-serine would be increased and so capacity to metabolize D-glycerate by D-glycerate kinase would be surpassed.

Among the four observations of D-glyceric acidemia previously described in the literature, no enzymatic lesion could be definitely elucidated. However, according to loading test results, various hypotheses were proposed concerning the possible enzymatic defect (see Table 1).

In conclusion, we suggest that D-glycerate excretion is a feature shared by several different metabolic disorders. It can be related to single or multiple enzymatic defects in either the fructose or the L-serine metabolic pathways, or both.

We thank Marie-Paule Delescat, Fabienne Hottewart, and Dominique Dillies for their excellent technical assistance.

References