Clinica Chimica Acta, 55 (1974) 41—47

CCA 6499

FRUCTOSE-1,6-DIPHOSPHATASE DEFICIENCY: ANOTHER ENZYME DEFECT WHICH CAN PRESENT ITSELF WITH THE CLINICAL FEATURES OF "TYROSINOSIS"

H.D. BAKKER, P.K. DE BREE, D. KETTING, F.J. VAN SPRANG and S.K. WADMAN

Wilhelmina Children's Hospital of the University of Utrecht, Nieuwe Gracht 137, Utrecht (The Netherlands)

(Received March 10, 1974)

Summary

An infant with a picture of hereditary liver disease corresponding in many respects with so-called "tyrosinosis" is described. The primary defect appeared to be fructose-1,6-diphosphatase deficiency, which was not recognized during the patient's life.

Many abnormalities of amino acid metabolism and transport occurred when the patient was on a diet containing saccharose. At that time tyrosyluria was excessive and serum tyrosine strongly elevated. Serum methionine was moderately increased, but a striking cystathioninuria was present and once even homocystine was traced in the urine. In the serum alanine was excessive, but also phenylalanine, lysine, proline, valine, threonine, leucine, serine and other amino acids were increased. There was a generalized amino aciduria and a massive lactic aciduria. Once even phenyllactic acid was found in the urine.

When the diagnosis "tyrosinosis" is proposed, fructose-1,6-diphosphatase deficiency as a primary cause should also be kept in mind.

Introduction

Many clinicians still consider "tyrosinosis" as a disease primarily due to a deficiency of *p*-hydroxyphenylpyruvatehydroxylase (EC 1.13.11.27). Clinically patients with "tyrosinosis" are characterized by liver disease, renal tubular defects, vomiting, diarrhoea and failure to thrive. Chemical abnormalities are tyrosinemia and increased urinary tyrosine, *p*-hydroxyphenyllactic acid (*p*-OHPLA), *p*-hydroxyphenylpyruvic acid (*p*-OHPPA), *p*-hydroxyphenylacetic acid (*p*-OHPAA) and *N*-acetyltyrosine. They also have a disturbed methionine metabolism, leading to hypermethioninemia. The metabolism of many other amino acids may be defective too, as well as their renal reabsorption. Finally, general chemical abnormalities accompanying liver and renal dysfunction are present.

However, serious doubt exists whether *p*-hydroxyphenylpyruvatehydroxylase deficiency is the primary cause of the condition [1—4]. Patients with a permanent tyrosinemia and tyrosyluria, but without liver and renal disease have been described [5—8]. In children with other primary enzyme defects a clinical picture similar to "tyrosinosis" can occur, e.g. in young patients with galactosemia due to a deficiency of galactose-1-phosphate uridylyltransferase (EC 2.7.7.10) [9]. Recently it was recognized that also in patients with hereditary fructose intolerance, due to a deficiency of fructose-1-phosphate aldolase (EC 4.1.2.7) the picture of "tyrosinosis" develops when they are nourished with a saccharose-containing formula [10—13]. Now we can add a deficiency of fructose-1,6-diphosphatase (EC 3.1.3.11) to the group of primary enzyme defects, which may be the cause of the clinical condition "tyrosinosis". In this paper we give the results of a retrospective study of a patient, described previously [14], who was admitted with a clinical picture responding in many respects to the criteria of "tyrosinosis". Initially the fructose-1,6-diphosphatase deficiency was not recognized. The abnormalities of the amino acid metabolism in this patient seemed to be connected with the dietary administration of saccharose.

Methods

Urinary amino acids shown in Table I were determined by the standard Technicon TSM 1 procedure for physiological fluids (retrospective analysis of samples stored at −15° to −20°). For serum amino acids (Table II) the data obtained previously by the Technicon NC I procedure are given (12.5 h, sodium buffers, 60°) [15]; glutamine and asparagine are lost in this method. *p*-Hydroxyphenyl acids, lactic acid and β-hydroxybutyric acid were determined by gas chromatography of their trimethylsilylated derivatives as described previously [16]. The following temperature program was used: 10 min at 75° followed by an increase of 2° per min up to 220° and finally 10 min at 220°.

Clinical course and results of laboratory investigations

The patient W.T.*, on admission a girl of 6 weeks, has been described briefly [14] with respect to her deficiency of fructose-1,6-diphosphatase (EC 3.1.3.11). She was the ninth child of healthy parents, who already had lost two other girls of 4 and 5 months. Both died of an unknown liver disease. The parents had one great-grandmother in common. This family history points to an inborn error of metabolism. Hepatomegaly, icterus, vomiting, diarrhoea, failure to thrive and hypotonia, combined with a marked tyrosinemia, tyrosyluria and hypermethioninemia, as well as overflow of many amino acids, suggested that the patient might be classified as a case of so-called "tyrosinosis". This supposition was still strengthened, when after institution of a diet on 19-4, in which the protein was partly replaced by a hydrolysate low in phenylalanine (30.5 mg/kg per day) and tyrosine (28.4 mg/kg per day), a striking diminution of the amino acid abnormalities followed within 1 week (see Tables I—III). The protein load was 2.0 g/kg per day.

---

* Dr H.A. Meurs, Juliana Ziekenhuis, Ede.

TABLE I

URINARY AMINO ACIDS (mg/g CREATININE) IN W.T., ON A SACCHAROSE-CONTAINING DIET ON 17-4 AND 13-8, AND ON A DIET WITHOUT SACCHAROSE ON 23-4, 6-5 AND 8-7-1968

Abbreviations: n, normal; pr, present; tr, trace.

	17-4	23-4	6-5	8-7	13-8
Hydroxyproline	748	62	109	< 8	172
Aspartic acid	778	tr	267	297	339
Threonine	5067	156	207	570	328
Serine	5956	424	315	701	900
Asparagine	3696	70	—	58	762
Glutamic acid	5289 }	45 }	355 }	370 }	25 }
Glutamine	4837	168	25	—	238
Proline	13089	161	112	< 6	204
Glycine	6919	503	711	1713	3000
Alanine	13304	524	342	549	2423
Citrulline	1711	—	—	pr	pr
α-Aminobutyric acid	3674	pr	pr	pr	—
Valine	1126	53	141	31	29
Cystine	1274	226	88	110	363
Methionine	n*	n*	n*	n	—
Cystathionine	1600	556	498	231	247
Isoleucine	244	tr	114	23	29
Leucine	548	148	299	45	49
Tyrosine	8370	12	72	70	641
Phenylalanine	1985	27	108	< 75	75
Homocystine	65	—	—	—	—
β-Amino isobutyric acid	194	tr	—	—	—
Tryptophan	207	—	—	—	—
Ornithine	129	pr	pr	pr	pr
Lysine	3896	271	482	127	455
Histidine	8341	1429	**	1172	3523
3-Methylhistidine	52	52	—	48	< 124
Carnosine	237	89	—	—	—
Arginine	333	63	241	26	79
Creatinine	135 mg/l	140 mg/l	110 mg/l	174 mg/l	130 mg/l

* Methionine on 17/4 not completely resolved but probably normal.
** Histidine on 6/5 probably not reliable.

Retrospectively, we conclude that the improvement was rather due to a replacement of the saccharose from the diet by lactose. Up until then, and also at home, she had a "Mulsoy" formula, corresponding to a saccharose intake of 9.2 g/kg per day. Before the diet change a severe lactic aciduria was also present, which, after removal of the saccharose, diminished drastically.

Despite the chemical normalization, however, her clinical condition did not improve very much: jaundice persisted (on 3-5 serum bilirubin was 9.3 mg/100 ml) and serum protein was only 3.9 g/100 ml. More natural protein was introduced up to a total load of 2.5 g/kg. Tyrosinemia and tyrosyluria did not reappear, which was interpreted as a proof of the absence of hereditary *p*-hydroxyphenylpyruvatehydroxylase deficiency. Then investigations were directed to carbohydrate metabolism without arriving at the proper diagnosis, however. When on 8-7 a saccharose containing formula was reinstituted (10.1 g/kg per day), diarrhoea became more frequent, serum bilirubin increased and so did G.P.T. A massive lactic aciduria occurred and on 16-8 serum lactic acid

TABLE II

SERUM AMINO ACIDS (mg/100 ml) IN W.T.

On 17-4 a saccharose-containing diet was given. On 19-4 saccharose was removed and protein was largely replaced by a hydrolysate low in tyrosine and phenylalanine up until 14-5; after that date a lactose-containing milk formula was given.

	17-4	23-4	29-4	6-5	17-5	8-7
Aspartic acid	0.44	0.09	0.13	0.19	0.26	0.44
Threonine	8.02	5.29	3.04	4.73	2.54	5.15
Serine	6.07	2.05	2.32	2.66	2.64	3.87
Citrulline	1.70	0.25	0.43	0.38	0.24	0.46
Proline	11.9	5.89	2.63	2.04	2.56	3.63
Glycine	3.51	1.58	1.82	1.91	1.66	2.10
Alanine	38.4	4.86	2.51	2.78	3.32	5.91
α-Aminobutyric acid	2.54	0.85	0.17	0.28	0.20	0.45
Valine	8.47	2.56	1.46	1.35	1.35	2.60
Cystine	1.56	0.64	0.60	0.85	0.71	—
Methionine	1.31	0.75	0.36	0.32	0.22	0.36
Cystathionine	0.45	—	—	—	0.11	
Isoleucine	2.63	0.80	0.45	0.34	0.52	0.78
Leucine	6.52	1.85	0.98	1.01	0.97	1.83
Tyrosine	23.3	1.34	0.30	0.41	0.72	0.90
Phenylalanine	10.1	0.50	0.27	0.37	0.37	0.69
Ornithine	3.14	1.07	0.99	0.97	0.89	1.39
Lysine	11.6	4.04	2.26	2.28	2.28	2.99
Histidine	4.44	2.56	1.61	1.15	0.83	1.43
Arginine	4.79	0.94	0.75	0.64	0.95	1.43

was higher than 200 mg/100 ml (blood pH 6.99; base excess −24 mequiv/l, despite bicarbonate therapy). The patient died the same day.

Autopsy revealed that the liver was enlarged. There was no cirrhosis or fibrosis, but fat accumulation was present. There was a slight inflammation of the periportal areas and the bile canaliculi were intact.

Table III shows pronounced abnormalities of the tyrosine, phenylalanine

TABLE III

URINARY AMINO ACIDS AND METABOLITES EXPRESSED AS mg/g CREATININE IN W.T. ON A SACCHAROSE-CONTAINING DIET
n, normal.

	17-4	13-8
Tyrosine	8370	641
p-OHphenyllactic acid	7630	5460
p-OHphenylpyruvic acid	296	—
p-OHphenylacetic acid	919	615
Phenylalanine	1985	75
Phenyllactic acid	274	—
Alanine	13304	2423
Lactic acid	30519	196900
Methionine	n	n
Homocystine	65	—
Cystathionine	1600	247
Cystine	1274	363
β-Hydroxybutyric acid	6519	4920

and methionine metabolism, as well as lactic acid and related products, on 17-4 and 13-8 when the diet contained saccharose. In the intermediate period, on a less harmful diet, metabolites were rather normal, except for cystathionine. On 17-4 and 13-8 there was an equal protein intake (4.5 g/kg per day), but diarrhoea and vomiting occurred, which makes comparison of the excretory levels rather difficult.

On 17-4 the excretion of *p*-OHPLA was as high as 7630 mg or 46 mmoles per g creatinine. For *p*-OHPPA and *p*-OHPAA lower values were found. A corresponding serum tyrosine of 23.3 mg/100 ml fits well in the picture, but urinary tyrosine (8370 mg or 46 mmoles per g creatinine) is higher than occurs in most patients with "tyrosinosis". This may point to a diminished tubular reabsorption of tyrosine. For amino acids as glycine, cystine, histidine and others, the same may be true.

On 17-4 the gas chromatogram also showed the presence of phenyllactic acid (274 mg or 1.65 mmoles per g creatinine) the identity of which was proved by gas chromatography—mass spectrometry. Phenylpyruvic and o-hydroxyphenylacetic acids were absent. The excretory pattern is different from that seen in phenylketonuric patients, who do not excrete phenyllactic acid at a serum phenylalanine concentration of 10 mg/100 ml. The urinary phenylalanine (1985 mg or 12 mmoles per g creatinine) is some tenfold higher than is seen in phenylketonurics with the same serum level of phenylalanine, indicating renal loss of this amino acid in W.T. On 13-8 no phenyllactic acid occurred and the increase of urinary phenylalanine was insignificant.

On 17-4 serum methionine was increased, but less than in most patients with "tyrosinosis". However, cystathioninuria was strongly increased and even homocystine was detected in the urine. Although serum methionine normalized gradually, urinary cystathionine remained abnormal, in spite of the removal of saccharose from the diet. Homocystine did not reappear in her urine after the introduction of saccharose. On 13-8 urinary cystathionine was much lower than on 17-4. The dietary methionine intake was comparable (62 compared with 68 mg/kg per day).

The exceptionally high concentration of serum alanine on 17-4 is not a feature of "tyrosinosis". It is probably connected with the overproduction of pyruvic acid. Urinary excretions of alanine and lactic acid were also massive when the saccharose-containing diet was given, but the ratio of urinary alanine and lactic acid was very different on 17-4 and 13-8, when approximately the same saccharose load per kg was given. We should take into account the fact that the clinical condition on 13-8 was quite different from that on 17-4 due to the manifest severe metabolic acidosis on 13-8.

It is amazing that during the first weeks of admission the lactic acidosis was not observed clinically; compensative dyspnea must have been absent. During the last days of her life, when lactic acid production was excessive, hyperventilation was quite pronounced.

Discussion

From the case history described above, it becomes clear that deficiency of *p*-hydroxyphenylpyruvatehydroxylase, underlying the tyrosinemia and tyro-

syluria, is not the primary defect. The striking coincidence of periodic amino acid anomalies and saccharose feeding points to fructose-1,6-diphosphatase deficiency. With such a defect present, daily administration of large amounts of saccharose may lead to accumulation of the intermediary metabolites fructose 1,6-diphosphate [17], fructose 1-phosphate [17] and their C-3-degradation products. In hereditary fructose-intolerance, fructose 1-phosphate accumulation induced by fructose has been shown in liver [18], kidney [19] and small intestine [20,21]. Such patients, when on a sacchrose-containing diet, also develop a generalized amino aciduria and elevated plasma amino acid concentrations, among others of tyrosine and methionine, as well as a striking tyrosyluria [9—12]. Clinically liver disease is manifest.

The patient described by us in many respects showed symptoms of the disorder which is called "acute tyrosinosis", recently reviewed by Goodwin [22]. The family history strongly pointed to hereditary liver disease. Prominent features were failure to thrive, vomiting, diarrhoea, lethargy, hepatomegaly and hypotonia. However, there was no cirrhosis; ascites and edema were absent and blood coagulation was normal. She had a persistent icterus, abnormal enzyme parameters indicative of liver disease and disturbances of amino acid metabolism, such as tyrosinemia/tyrosyluria and cystathioninuria. There were signs of a diminished renal amino acid reabsorption, but hypophosphatemic rickets and mellituria were lacking. After the diet had been changed as described above, the chemical improvement was very suggestive.

The symptoms of patients with fructose-1,6-diphosphatase deficiency have been described by several authors [14,23—27]. In our case the symptoms of fructose-1,6-diphosphatase deficiency were rather dubious: clinically no signs of metabolic acidosis, no hypoglycemia, probably due to frequent saccharose-containing meals (10 per day) and even an unexpected hyperglycemic reaction on a fructose load, which might reflect phosphorylation of fructose via the hexokinase reaction [14,28]. The fact that an excessive load of fructose as large as 3 g/kg was given (and not 2 g, as was written erroneously [14]) may further support this hypothesis.

When patients as described are presented for screening for inborn errors of amino acid metabolism, a strongly increased alanine concentration of the serum should have the investigator's special attention. Then gas chromatography of trimethylsilylated non-volatile acids is indicated and will lead to the detection of lactic acidosis. Whether or not the diet contains saccharose is an anamnestic item, which can contribute to the interpretation of the amino acid anomalies.

References

1 B.N. La Du and L.R. Gjessing, in J.B. Stanbury, J.B. Wyngaarden and D.S. Frederickson (Eds), The metabolic basis of inherited disease, McGraw-Hill, New York, 3rd edn, 1972, p. 296
2 G.E. Gaull, D.K. Rassin, G.E. Solomon, R.C. Harris and J.A. Sturman, Pediat. Res., 4 (1970) 337
3 W. Nützenadel, P. Lutz and H. Bickel, Z. Kinderheilk., 113 (1972) 193
4 H.D. Bakker, Tyrosinosis, tyrosinemie en tyrosylurie, Thesis, Utrecht, 1973
5 G. Medes, Biochem. J., 26 (1932) 917
6 S.K. Wadman, F.J. van Sprang, J.W. Maas and D. Ketting, J. Ment. Def. Res., 12 (1968) 269
7 A. Hill, Clin. Biochem. J., 4 (1971) 263
8 J.L. Holston, H.L. Levy, G.A. Tomlin, R.J. Atkins, T.H. Patton and T.S. Hostly, Pediatrics, 48 (1971) 393

9 M.M. David, C. Collombel, J.C. Dutruge and M. Jeune, Pédiatrie, 25 (1970) 459
10 D.B. Grant, F.W. Alexander and J.W.T. Seakins, Acta Paediat. Scand., 59 (1970) 432
11 R. Lindemann, L.R. Gjessing, B. Merton and S. Halvorssen, Lancet, i (1969) 391
12 R. Lindemann, L.R. Gjessing, B. Merton, A.C. Löken and S. Halvorssen, Acta Paediat. Scand., 59 (1970) 141
13 C. Willems, A. Heusden, P. Renson, C. Leyat, Y. Monard and L. Stainier, Helv. Paediat. Acta, 26 (1971) 467
14 W.C. Hülsmann and J. Fernandes, Pediat. Res., 5 (1971) 633
15 G.J. van Stekelenburg, Technicon Symposia, II (1966) 445
16 S.K. Wadman, C. van der Heiden, D. Ketting and F.J. van Sprang, Clin. Chim. Acta, 34 (1971) 277
17 E.R. Froesch. in J.B. Stanbury, J.B. Wyngaarden and D.S. Frederickson (Eds), The metabolic basis of inherited disease, McGraw-Hill New York, 3rd edn, 1972, pp. 144—145
18 H.G. Hers and G. Joassin, Enzymol. Biol. Clin., 1 (1961) 4
19 R.C. Morris, I. Ueki, D. Loh, R.Z. Eanes and P. McLin, Nature, 214 (1967) 920
20 J. Nisell and L. Linden, Scand. J. Gastroenterol., 3 (1968) 180
21 E.R. Froesch, in J. B. Stanbury, J.B. Wyngaarden and D.S. Frederickson (Eds), The metabolic basis of inherited disease, McGraw-Hill, New York, 3rd edn, 1972, p. 137
22 B.L. Goodwin, in Tyrosine Catabolism, Clarendon Press, Oxford, 1972, p. 43
23 L. Baker and A.J. Winegrad, Lancet, i (1970) 13
24 K. Baerlocher, R. Gitzelmann, R. Nüssli and G. Dumermuth, Helv. Paediat. Acta, 26 (1971) 489
25 A.S. Pagliara, I.E. Karl, J.P. Keating, B.I. Brown and D.M. Kipnis, J. Clin. Invest., 51 (1972) 2115
26 S.B. Melancon, A.K. Khachadurian, H.L. Nadler and B.I. Brown, J. Pediatr., 82 (1973) 650
27 J.M. Saudubray, J.C. Dreyfus, C. Cepanec, H. Le Lo'ch, Pham Hu Trung and P. Mozziconacci, Arch. Franc. Pédiatr., 30 (1973) 609
28 B.R. Landau, J.S. Marshall, J.W. Craig, K.Y. Hostetler and S.M. Genuth, J. Lab. Clin. Med., 78 (1971) 608