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Thiamine Dependency in a Patient with Congenital Lacticacidaemia Due to Pyruvate Dehydrogenase Deficiency[1]

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Abstract

A patient with congenital lactic acidosis, muscular hypotonia and severe ataxia is reported. The aetiology of his disease was found to be a deficiency of pyruvate dehydrogenase (E.C. 4.1.1.1). Thiamine treatment (1.8 g/day) was successful in correcting biochemical and clinical symptoms. The mechanism of its action is probably based on activation of pyruvate dehydrogenase through interference in the physiologic regulation.

LONSDALE [1] reported a patient with congenital lactic acidosis, optic atrophy and intermittent episodes of ataxia. During such episodes, high concentrations of lactate, pyruvate and alanine were found. A deficiency in the decarboxylation of pyruvate was postulated but not proven. The encephalopathy appeared to be similar to that described by WERNICKE [2] which is associated with a deficiency of thiamine intake. Therefore 600 mg of thiamine per day was given to this patient. The authors stated: 'Urinary pyruvate and alanine concentrations have since decreased and the boy has remained well'. There is nothing known about the further course of this patient.

Since 1971 several patients have been described with proven pyruvate dehydrogenase (PDH) complex deficiencies (see Table 1). Thiamine therapy has been tried in some, but there have been no reports of clinical outcome being definitely improved.

This paper reports a patient with PDH deficiency who responds very favourably to the application of high doses of thiamine, the beneficial effect has now lasted three years.

Case report

R.Z. is the second child of healthy parents. His psychomotoric development seemed to be normal up to the age of 13 months when his mother noted that he did not try to stand up and that he did not babble as his healthy older sister had done at the same age. At 20 months R.Z. was referred to our hospital because of his marked psychomotoric retardation. The main clinical feature was severe muscular hypotonia. The boy could neither stand nor walk. No discrete abnormal neurological signs were found. The development quotient according to Bühler-Hetzer was 64. The EEG was normal for his age. In our metabolic screening program alanine concentration in the urine appeared to be high (pherogram obtained by high voltage electrophoresis and stained by ninhydrin [16]). This led to the examination of ammonia, lactate and pyruvate concentrations. Ammonia concentration in plasma was normal (35 μmol/l). In whole blood lactate and pyruvate concentrations were elevated (lactate concentration: 3 mmol/l, reference values (rv): 1–1.8 mmol/l; pyruvate concentration: 170 μmol/l, rv: 40–67 μmol/l). The ratio lactate conc./pyruvate conc. was 17.6 (rv: 10–20). Arterial pO_2 was within normal limits. Several clinical tests were performed to elucidate the nature of this lacticacidaemia. A definite diagnosis could not be established at this time. As a minor deficiency of PDH could not be ruled out, a therapeutic experiment with high doses of thiamine (3 × 600 mg/day) was started. Within the next 6 months, R.Z. began to stand up, to walk and to speak. His mother was delighted. As we were not convinced of a causal relationship between thiamine therapy and this progress, we stopped medication. This was done at home. Promptly the boy became severely atactic. He kept falling down 'all the time' so that finally he did not even try to stand up again. His

[1]) Dedicated to K. Bucher on the occasion of his 65th birthday.

Table 1
Patients with PDH deficiency

Author	Deficient enzyme (per cent activity of corresponding controls)	Patient clinical state	Thiamine therapy	Effect	Further clinical course
J.P. Blass et al. [3, 4]	PDH: E.C. 4.1.1.1, 17%	boy, 5 yr., intermittent ataxia lasting hours to one month	600 mg/d	attack frequency reduced?	not stated (n.s.)
J.P. Blass et al. [5]	PDH: 15% (E.C. 2.3.1.12 or E.C. 1.6.4.3)	girl, 3 yr., severely retarded, microcephaly, optical atrophy	600 mg/d	no effect	n.s.
J.L. Willems et al. [6]	PDH: E.C. 4.1.1.1, 10%	boy, 9 m., mental retardation, microcephaly	500 mg/kg × d	concentration of pyruvate lowered no clinical effect	died at 12 m.
D.F. Farrell et al. [7]	PDH: E.C. 4.1.1.1, 0%	boy, neonate, severe clinical course (no details given)	–	–	died at 6 m.
B.H. Robinson and W.G. Sherwood [8]	PDH phosphatase	boy, neonate, intermittent exacerbations of acidosis	dosage not stated	no effect	died at 6 m.
Y. Oka et al. [9]	PDH: E.C. 4.1.1.1	boy, 5 yr., intermittent sopor, ataxia and vomiting	200 mg for 2 days	no effect	n.s.
S.D. Cederbaum et al. [10]	PDH: E.C. 2.3.1.12 (?), 15%	boy, 8.5 yr., profoundly retarded, microcephaly choreoathetoid (girl, sibling, died at 7 yr.: choreoathetose; girl, sibling 3.5 yr., choreoathetoid, optical atrophy)	300 mg/d	no effect	n.s.
J.H. Strömme et al [11]	PDH: E.C. 4.1.1.1, 10%	girl, neonate, small for date, respiratory failure, flaccid	–	–	died at 3 weeks
J.P. Blass et al. [12]	PDH and KGDH: (E.C. 1.6.4.3. ?), 43%, 50%	5 patients with Friedreich ataxia	–	–	n.s.
J.C. Haworth et al. [13]	PDH and KGDH, 13%, 39%	3 siblings: boy, neonate, severe retardation (premature)	45–100 mg/d	no effect	died at 3½ m.
		girl, neonate (2.6 kg), attacks of acidosis			died at 4½ m.
		boy, neonate, intermittent seizures microcephalic, mild retard			at age 23 months development quotient 45–55 (DQ)
R.E. Falk et al. [14]	PDH: E.C. 4.1.1.1, 20%–12%	2 brothers: 11 yr. and 2 yr. 3 m., psychomotoric retardation, episodes of ataxia, ophthalmoplegia	–	–	better with ketogenic diet
H. Wick [15]	PDH: E.C. 4.1.1.1, 2%	girl, neonate, small for date, agenesia of corpus callosum, acidosis, respiratory failure	100 mg/kg × d	no effect	died at 2 weeks
H. Wick	PDH: E.C. 4.1.1.1	boy, 20 months, retarded (development quotient: 64) severe muscular hypotonia and ataxia (patient R.Z.)	1800 mg/d	clinical and biochemical effect	at age 3 yr. only slight ataxia normal muscular tonus, development quotient: 90

mother was frightened and brought him to the hospital. After recommencement of thiamine therapy the ataxia disappeared within 2 days.

At the age of 3 years the patient was admitted for a short clinical check-up. The development quotient now was 90. In the neurological examination a normal muscular tonus was found. Slight ataxia still persisted. Lactate (1.4 mmol/l) and pyruvate (56 μmol/l) were within normal limits.

At the age of $3\frac{1}{2}$ years in a phase of aggression he obstinately refused to take his tablets. Instead of 6 tablets (300 mg thiamine each) he received only an uncontrolled amount each day. He again became severely atactic. His mother felt very guilty and did not dare to consult the doctor. Only when she noted a strabismus did she give him the tablets by force. Again the ataxia subsided, but a slight strabismus remained. The ophthalmologic diagnosis was: strabismus concomitans convergens due to refractory anomaly. Since then the boy wears glasses and the strabismus has disappeared. The possible relationship between his strabismus and this episode of ataxia is not clear.

Methods

Estimations of lactate, pyruvate, glucose and fructose were performed by standard methods of clinical chemistry. Ammonia concentration in plasma was measured with the method of Fenton [17] adapted to the use of 0.1 ml of

plasma by substituting a batch procedure for the original column chromatography. Alanine concentrations were estimated by automated column chromatography (with a Biocal BC 200).

CO_2 production from $^{14}C_1$-pyruvate by intact fibroblasts as well as PDH activity (E.C. 4.1.1.1) were measured according to BLASS et al. [4]. PDH complex activity was measured by the method of BLASS et al. [12, 18].

Fibroblast cultures were grown in Hams Medium F 10 (modified: Flow Laboratories). Preincubations of fibroblast cultures were done with medium containing final concentrations of thiamine of 400 mg/l or 1200 mg/l (time of preincubation see Table 5).

Puromycin, 50 μg/ml, added to the incubation medium was used for inhibition of protein synthesis.

Results

Clinical investigations

The following tests are helpful in the differentiation of lactic acidosis.

1. Glucagon test. A normal rise of glucose plasma concentration after administration of glucagon rules out glucose-6-phosphatase deficiency which is known to be associated with lactic acidosis. 0.3 mg Glucagon was administered intravenously. Glucose concentration in plasma rose within 15 minutes from 3.6 to 4.7 mmol/l (low normal response). Lactate conc. rose from 2.3 to 4.4 mmol/l. This value was reached after 45 minutes.

2. Fructose load. Deficiency of fructose-1,6-phosphatase, a key enzyme of gluconeogenesis, is another cause of lactic acidosis. Fructose (0.5 g/kg body weight) is given intravenously within 3 minutes. The normal rise in glucose concentrations in plasma obtained in our patient rules out such a deficiency. At the same time this test helps to discriminate between 'physiologic' elevations of lactate concentrations (due to muscular activity) and hyperlacticacidaemia (due to a metabolic disorder). See Figure 1.

3. Glucose load. Glucose (0.5 g/kg body weight i.v.) application may help to diagnose PDH deficiencies. Lactate concentrations will rise significantly if utilization of pyruvate is impaired. This test is suitable for judging the effect of a therapeutic agent on lacticacidaemia. See Figure 2.

4. Alanine load. Alanine (0.3 g/kg body weight in 5 minutes) is used for synthesis of glucose provided that liver glycogen has been depleted: when starting the test the patient must have fasted to a mildly hypoglycaemic state. If this is not the case, a second effect of alanine, i.e. stimulation of glucagon secretion and a consecutive rise of glucose concentration in plasma, can be falsely interpreted as a normal functioning of gluconeogenesis [19]. Figure 3 demonstrates the normal rise of glucose concentration from a hypoglycaemic starting point after the alanine load.

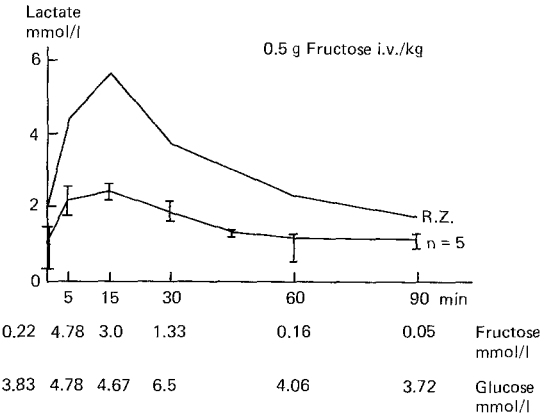

Figure 1
Fructose load (0.5 g/kg body weight i.v.) The lower curve represents mean values and range of lactate concentrations in 5 normal children (2–5 years of age), performed in order to exclude a suspected fructose intolerance.

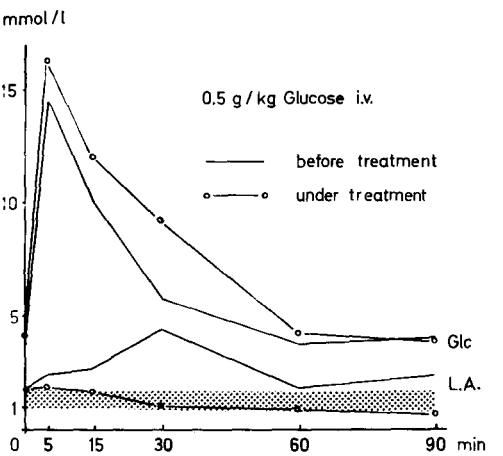

Figure 2
Glucose load (0.5 g/kg body weight i.v.). The course of lactate concentrations has been normalized under thiamine treatment.

Investigations with fibroblasts

The results of investigations with fibroblasts appear in the following tables (Tables 2, 3 and 4).

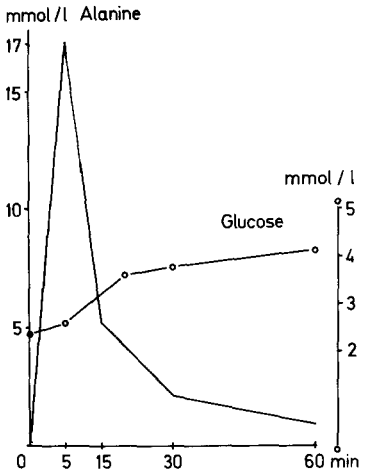

Figure 3
Alanine load (0.3 g/kg body weight i.v.). Fasting hypoglycaemia is corrected by alanine administration.

Table 2
CO_2 production from $^{14}C_1$-pyruvate in intact fibroblasts.

		n[a]	pmol/min × mg protein (Mean ± SD)	p (t-test)
No addition of thiamine HCl	R.Z.	7	39 ± 23	<0.001
	r.v.	20	280 ± 91	
With addition of thiamine HCl	R.Z.	6	97 ± 52	<0.005
	r.v.	11	261 ± 52	

[a] n = number of estimations.

Table 3
Activity of PDH complex.

	n	pmol/min × mg protein (Mean ± SD)	p (t-test)
R.Z.	7	8.0 ± 2.6	<0.001
r.v.	21	18.2 ± 6.4	

Table 4
Activity of PDH (E.C. 4.1.1.1).

	n	pmol/min × mg protein (mean ± SD)
R.Z.	3	4.5, 9.8, 11.5
r.v.	22	21.3 ± 7.8

Discussion

About 15 patients with a proven deficiency of PDH have been published up to now (see Table 1). Thiamine therapy has been tried in a few cases. A positive effect has only been reported in two (see Refs. [3] and [6]). One patient received 600 mg/day, a pharmacological dose, the other one the excessively high dose of 500 mg per kg body weight per day. The improvement noted in the latter patient was limited to a reduction of pyruvate and presumably lactate concentrations. The clinical course was not altered. The patient died at 12 months.

As our patient appears to be the first one who really profited from thiamine therapy for a prolonged period, we tried to obtain some information concerning the mechanism of this beneficial effect. Table 4 shows that the deficiency of pyruvate utilization in the fibroblasts of R.Z. can be restricted to PDH (E.C. 4.1.1.1), the first enzyme of the PDH complex. In this first step pyruvate is decarboxylated and the remaining C_2 fragment is attached to thiamine pyrophosphate (TPP) – the true coenzyme of PDH (E.C. 4.1.1.1). The product, 2-oxyaethylthiamine pyrophosphate, interacts directly with the second enzyme of the complex, dihydrolipoyltransacetylase (E.C. 2.3.1.12) and TPP is regenerated.

In intact fibroblasts CO_2 production from pyruvate is known to be enhanced by thiamine [2] the precursor vitamin of TPP. Table 2 shows that this effect is clearly demonstrable in the cell lines from R.Z. (the reference values are somewhat misleading; if simultaneous experiments are performed in control fibroblasts comparing CO_2 production with or without addition of thiamine, the enhancing effect of thiamine is reproducible and lies between 20 and 70%). This probably rules out a disorder of TPP formation in the patient.

Preincubations with very high concentrations of thiamine for different periods were used in the experiments in Table 5. A marked increase of enzymatic activity was achieved by this procedure in control fibroblast cultures as well as in the fibroblast cultures of R.Z. This effect is time dependent, maximal activities are reached after 3 to 5 hours of pretreatment. The possibility of enzyme induction was considered. In one preliminary experiment with the cells of R.Z. puromycin was added as an inhibitor of protein synthesis. This had no effect on the enhancement of enzyme activity. It can be concluded that this increase in activity is prob-

Table 5
Preincubation of fibroblasts with thiamine: PDH complex activity (in pmol/min × mg protein)

	Fibroblasts in standard medium	Fibroblasts in medium with added thiamine				
		400 mg/l				1200 mg/l
		(time in hours) 1	3	5	24	60
Reference cell lines						
Experiment 1	20 (3)[a]					25 (3)[a]
2	13 (3)		34 (3)	38 (3)	41 (3)	37 (3)
Cell lines of R.Z.						
Experiment 1	9.0 (3)					13.7 (3)
2	7.25 (4)	8.3 (3)	10.5 (2)	20 (3)		
3	9.7 (2)			16.6 (3)		
				17.1 (3)[b]		

[a] Number of estimations in parentheses.
[b] Addition of 50 μg/ml Puromycin during pretreatment with thiamine HCl.

ably due to activation of the PDH complex rather than due to induction.

PDH activity is controlled by an ATP-dependent PDH kinase which phosphorylates PDH and thus inhibits its activity, and a PDH phosphatase which restores activity [20].

ROCHE and REED [21] showed that TPP inhibits phosphorylation of PDH, maintaining PDH in its active form. HOMMES [22] studied the effect of 125 mg/kg thiamine given intraperitoneally to rats for 4 days. This treatment resulted in a decreased sensitivity of PDH to inhibition by ATP. His results indirectly confirm the work of Roche and Reed in a totally different experimental set-up. The results of Table 5 (enhancement of PDH activity after preincubation with high concentrations of thiamine) can be explained by the same mechanism, i.e. high intramitochondrial concentrations of TPP keeping PDH in its active form by inhibiting the phosphorylation mechanism.

In conclusion we assume that the PDH activity in the tissues of our patient is so much impaired that an impressive clinical disease results. Yet the residual activity is still high enough that interference with the physiologic regulatory process by thiamine therapy enhances pyruvate utilization sufficiently to enable the patient to lead a normal life with no biochemical abnormalities and no clinical symptoms detectable apart from mild residual ataxia. This interference in an important physiologic regulation by treatment might well have some drawbacks, but up to now the obvious beneficial effects of therapy outweigh by far these negative theoretical considerations.

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