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Dihydropyrimidine dehydrogenase deficiency leading to thymine-uraciluria. An inborn error of pyrimidine metabolism

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Summary

Three unrelated patients with excessive thymine-uraciluria due to dihydropyrimidine dehydrogenase deficiency are described. Excretory values (mmol/g creatinine) were: uracil 2.0–10.5, thymine 2.3–7.5, 5-hydroxymethyluracil 0.2–0.9.

Orally administered (index patient) uracil and thymine were excreted for the greater part whilst dihydrouracil and *S*-dihydrothymine were mainly metabolised.

Dihydropyrimidine dehydrogenase activities (nmol $\cdot h^{-1} \cdot mg^{-1}$ protein) in leucocytes were 0.04, 0.01 and < 0.01 in the patients, 0.31–1.66 in their parents, and 1.01–4.46 in controls ($n = 4$).

The patients presented with a non-specific clinical picture of cerebral dysfunction.

Introduction

Recently a patient with developmental problems, and a persistent urinary excretion of excessive amounts of uracil, thymine and 5-hydroxymethyluracil (5-HMU) was presented [1,2]. Such a metabolite profile is suggestive of a deficiency of dihydropyrimidine dehydrogenase (dihydrouracil dehydrogenase ($NADP^+$)) (EC 1.3.1.2), an enzyme which catalyses the hydrogenation of both uracil and thymine.

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The scheme for the metabolism of both pyrimidines is depicted in Fig. 1. The results of oral loading and subsequent urine analysis supported this hypothesis. Administered uracil and thymine were mainly excreted unaltered, whilst their dihydro analogues were metabolised for a large part.

In the present paper we give a brief description of our index case and introduce

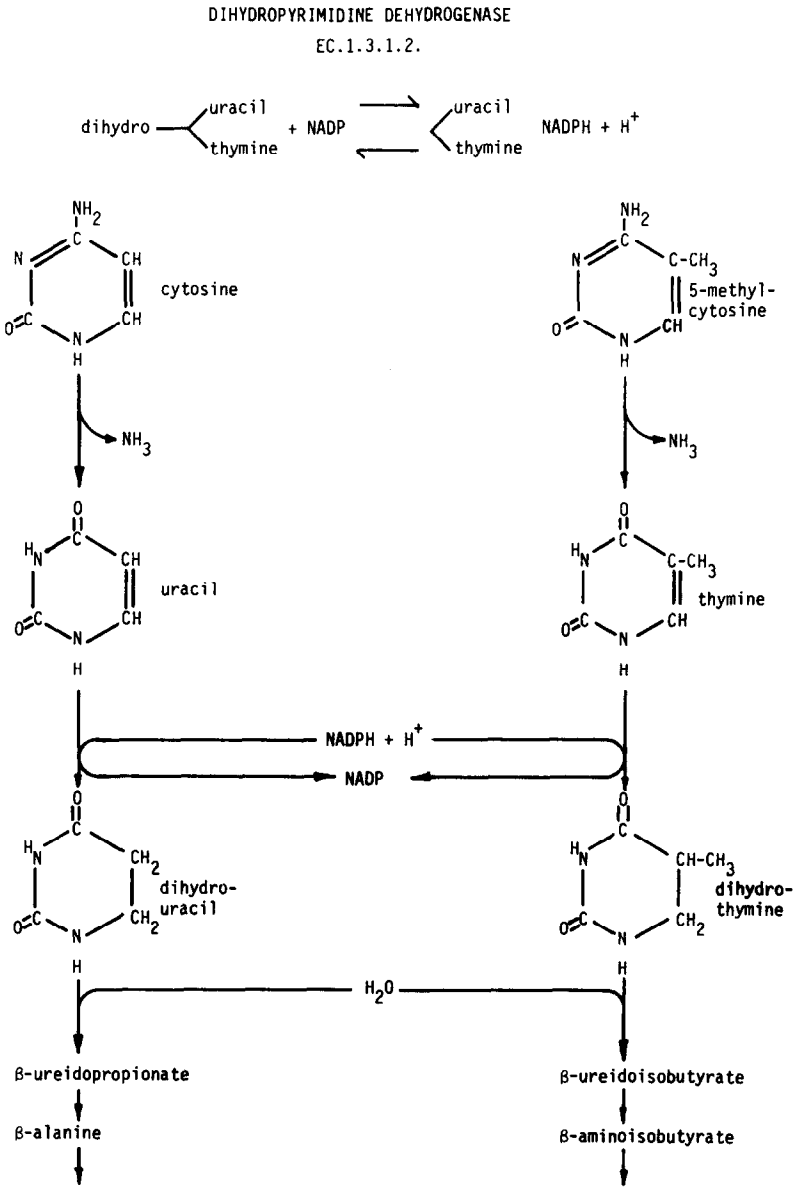

Fig. 1. The catabolism of uracil and thymine.

two new, unrelated patients. Quantitative information on their typical urinary metabolites is given. The determination of the proposed enzyme defect in leucocytes of all three patients is described and enzyme activities in all obligate heterozygotes are presented.

Short case reports

(1) Index patient R.E., a boy born 8th July, 1975, is the third child of healthy, unrelated parents. The first child is healthy, the second one died of perinatal asphyxia. His psychomotor development was normal until the age of $1\frac{1}{2}$ years. Then transient petit mal seizures occurred for 1 month. From the onset of the seizures the parents noticed behavioural changes. At the age of 4 years he was admitted to hospital because of developmental problems. Speech development had been retarded and his behaviour became solitary. There were no physical abnormalities. Autistic features were present but intelligence was normal. To date the patient's clinical condition is unremarkable; the only problem is his slightly abnormal behaviour.

(2) M.S., a girl born 13th October, 1969, is the only child from parents who are first cousins. Her neonatal course and early childhood were unremarkable. She went to infant class when she was 4 years old. Then her only problem was solitary behaviour. Between the age of 5 and 6 she developed absences which proved to be of epileptic origin.

At the age of 14 years her biochemical abnormality was demonstrated when her urine was subjected to gas chromatographic screening for inborn errors. Clinical observation revealed mental retardation (for which no perinatal cause could be sustained), epilepsy and hypohydrotic ectodermal dysplasia. Incidentally plasma uric acid was increased.

(3) B.B., a Turkish boy born 24th December, 1981, was admitted at the age of 9 months for diarrhoea and feeding difficulties. The child recovered after the introduction of diluted feeds followed by gradual re-introduction of normal nutrition. At the age of 15 months a severe growth retardation was observed. Length, skull circumference and weight were below the 10th percentile; bone age was 9 months. Urine screening for inborn errors revealed thymine-uraciluria (detected by 2-dimensional thin layer chromatography). To date his motor development is normal but mentally the child seems to be retarded. Growth retardation and microcephaly are persistent but it should be noted that both parents are short in stature.

Methods

Uracil and thymine were extracted from the acidified urine with ethylacetate (at a low extraction yield) and detected as their trimethylsilyl (TMS) derivatives by gas liquid chromatography (GLC) and mass spectrometry (GLC-MS) screening procedures [3]. Retention times (min) were: 35.89 for uracil; 37.79 for 2-phenylbutyrate (internal standard); 40.00 for thymine.

High resolution 2-dimensional thin layer chromatography [4,5] was also used for detection. For identification pyrimidines were isolated from the thin layer chromatograms, trimethylsilylated and their TMS-derivatives identified by GLC-MS.

Quantitative analysis of urinary pyrimidines was done with automated cation exchange chromatography and high pressure liquid chromatography [2,6,7]. Synthetic dihydrouracil (Sigma, St. Louis, MO, USA) and DL-dihydrothymine (Sigma) were hydrolysed in 6 mol/l HCl for 48 h at 150 °C and determined as β-alanine (β-Ala) (yield 77%) and β-aminoisobutyric acid (β-AIB) (yield 72%) by automated amino acid column chromatography on a cation-exchange resin (Technicon TSM 1® amino acid analyzer). Urinary dihydrouracil and dihydrothymine were comprised in fraction 3 and 4 obtained with the prefractionation procedure as described in [4]. These fractions were hydrolysed and β-Ala and β-AIB were determined in the hydrolysate. *R*- and *S*-enantiomers of β-AIB were determined as described in [8].

Dihydropyrimidine dehydrogenase in leucocytes was measured as described by Piper et al [9], a radiochemical method involving production of [methyl-^{3}H]dihydrothymine from [methyl-^{3}H]thymine. After incubation, precursor and product were separated by paper chromatography and radioactivity of the fractions counted.

Leucocytes were prepared from heparinised blood by the dextran sedimentation method. Fibroblasts were grown to confluence and harvested by standard methods.

Results

All three patients showed a nearly identical urinary pyrimidine profile (Table I). In addition to excessive amounts of uracil and thymine substantial quantities of 5-HMU, a metabolite of thymine, also were excreted. This compound is easily detected on a thin-layer chromatogram, but it is not observed in the gas chromatogram because of a low extraction yield with ethylacetate. Iso-orotic acid, another possible thymine metabolite, was absent. In Fig. 2 a typical 2-dimensional chromatogram is shown. Fig. 3 gives the mass spectrum of the TMS-derivative of 5-HMU. Loading tests were only performed in index patient R.E. (see Table II). The major part of the orally administered uracil and thymine was excreted unchanged in the first 24 h after loading, suggesting a severe dihydropyrimidine dehydrogenase deficiency. On the contrary only 7% of dihydrouracil was excreted in the first 24 h

TABLE I

Urinary uracil, thymine and 5-hydroxymethyluracil (mmol/g creatinine) in R.E., M.S. and D.B.

	Date of collection	Uracil	Thymine	5-Hydroxymethyluracil
R.E.	16-08-78	4.6	3.0	0.2
	07-04-82	2.4	2.3	0.4
M.S.	10-05-83	2.0	2.4	0.3
B.B.	18-07-83	10.5	7.5	0.9
Controls ($n = 6$)		0.07–0.30	N.D.	N.D.

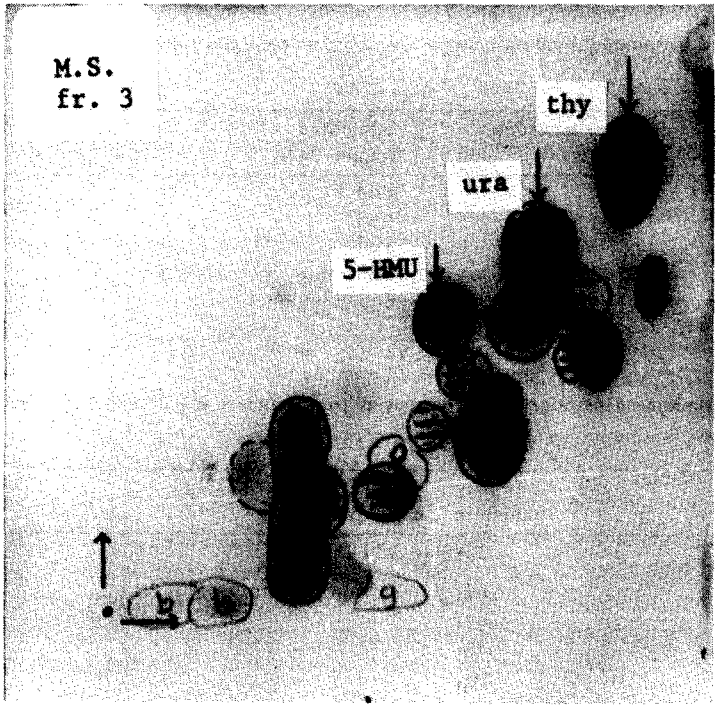

Fig. 2. Two-dimensional thin layer chromatogram of urinary pyrimidines and purines according to [4] (Patient M.S.).

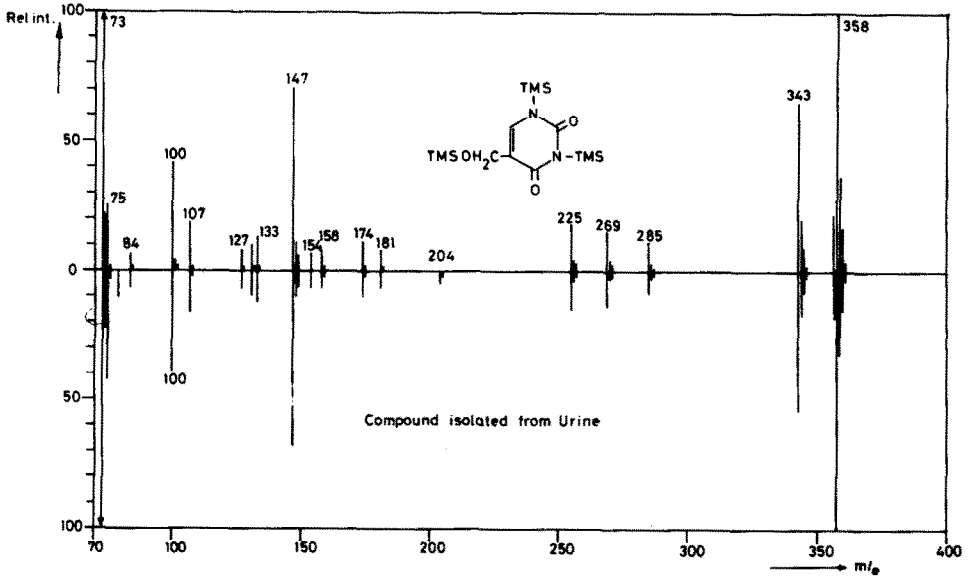

Fig. 3. Mass spectrum of synthetic 5-hydroxymethyluracil (Sigma) and that of compound isolated from urine (Patient R.E.).

TABLE II

Urinary excretion of uracil, thymine + 5-hydroxymethyluracil, dihydrouracil and dihydrothymine after oral loading in patient R.E.

Compound	Administered dose (mmol)	Recovered (mmol/24 h)	% of load
Uracil	11.9	8.84	74
Thymine + 5-HMU	11.9	8.65	73
Dihydrouracil	14.6	1.04	7
Dihydrothymine (*R*,*S*)	14.0	5.56	40

TABLE III

The activity of dihydropyrimidine dehydrogenase in leucocytes from patients R.E., M.S., B.B., their parents and controls

	Activity ($nmol \cdot h^{-1} \cdot mg^{-1}$ protein)
Controls $n = 4$	2.27 (range 1.01–4.46)
R.E.	0.04
Father	1.43
Mother	1.45
Healthy sibling	1.66
M.S.	0.01
Father	0.69
Mother	0.62
B.B.	< 0.01
Father	0.87
Mother	0.31

after loading with this compound. After loading with dihydrothymine, which was an equimolar mixture of the *R*- and *S*-form, 40% was recovered. After dihydrothymine loading β-AIB was excreted, the molar amount being 6.4% of the dihydrothymine intake; 95% of this urinary β-AIB had the *R*-configuration.

Dihydropyrimidine dehydrogenase activity was measured in leucocytes from patients, obligate heterozygotes and controls. The results are shown in Table III. All three patients had an almost complete absence of enzyme activity. The parents showed values intermediate between those of the patients and the controls. In control leucocytes the mean enzyme activity was 2.27 $nmol \cdot h^{-1} \cdot mg^{-1}$ protein. This value is in agreement with the activity reported by Piper et al [9] for transformed human lymphoblasts.

Discussion

Thymine-uraciluria can be detected by GLC-screening of urinary organic acids, using ethylacetate extraction and trimethylsilylation. Peaks are rather small, due to

the low extraction yield and can easily be overlooked. Confirmation by GLC-MS is essential.

Detection by 2-dimensional thin layer chromatography is facilitated by the fact that the chromatographic profile is characteristic and recognisable at first sight.

From the results obtained in the patients we conclude that all three had a generalised deficiency of dihydropyrimidine dehydrogenase. This follows from the highly elevated basal excretory values of uracil and thymine as well as from the loading tests proximal and distal to the enzyme block in the index patient. The deficiency has been proven explicitly in leucocytes, but from the metabolic abnormalities it can be presumed that the enzyme is deficient also in the liver, the main site of pyrimidine metabolism [10]. The enzyme values obtained from the leucocytes of the parents were intermediate, indicating heterozygosity and an autosomal recessive mode of inheritance.

Thymine-uraciluria has been described before in a 2-year-old child with a malignant tumour of the brain [11]. These authors concluded that the pyrimidines originated from the tumour because there was a correlation between their excretion rates and the clinical progress of symptoms, remission after therapy and relapse. However, dihydropyrimidine dehydrogenase determinations in fibroblasts revealed values as high as 50% of that of control fibroblasts. The spectrophotometric method used by these authors to determine the activity of dihydropyrimidine dehydrogenase is suitable for the purified enzyme. In crude homogenates one might overestimate the enzyme activity due to the high blank values [12]. Using the method of Piper et al we could not detect enzyme activity in control fibroblast homogenates prepared from $4–5 \times 10^6$ cells. This might indicate that the enzyme activity in fibroblasts is less than 5–10% of that in leucocytes. An alternative explanation for the thymine-uraciluria in the Swedish patient may be a generalised dihydropyrimidine dehydrogenase deficiency such as that in our patients. The fluctuations of the metabolite excretion in the Swedish child might have reflected the degree of catabolism associated with the tumour.

Thymine and uracil are excreted in roughly the same amounts. This phenomenon cannot be understood on a basis of catabolism of both endogenous and exogenous nucleic acids. The amounts of thymine containing DNA and *m*-RNA is by far smaller than the amount of RNA which does not contain thymine. The phenomenon could be explained in part by a higher re-utilisation of uridine compared to that of thymidine, resulting in a relatively low conversion of uridine to uracil.

Apart from the three patients described here, we know about two other patients. One of them is a child with severe neurological symptoms [13] and the other a baby with neurological problems and liver disease who died at early age [14]. It seems that dihydropyrimidine dehydrogenase deficiency presents with a non-specific clinical picture of cerebral dysfunction.

Acknowledgement

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