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Dihydropyridimidinase deficiency and congenital microvillous atrophy: Coincidence or genetic relation?

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Summary: We describe a boy of consanguineous parents who suffered from intractable diarrhoea due to congenital microvillous atrophy, a recessively inherited autosomal disorder. He developed severe cholestatis starting at 2 weeks of age and leading to liver cirrhosis. His psychomotor development appeared only slightly delayed. At the age of 7 months he died due to septicaemia. In addition to disturbances of electrolyte balance and renal tubular function, which could be attributed to microvillous atrophy, marked elevations of dihydouracil and dihydrothymine as well as moderately elevated excretion of uracil and thymine in urine were repeatedly demonstrated, suggesting a disorder of pyrimidine degradation. An enzymatic defect of 5,6-dihydropyrimidine amidohydrolase (EC 3.5.2.2, dihydropyrimidinase, DHP) was demonstrated in liver biopsy. As both of these recessive disorders seem to be extremely rare, it remains speculative, whether he suffered from two independently inherited metabolic diseases or whether this represents a hitherto undescribed contiguous gene syndrome.

Congenital microvillous atrophy (McKusick 251850) was recognized as a recessively inherited disorder in 1978 (Davidson et al 1978). A survey of 23 cases in 1992 (Phillips and Schmitz 1992) delineates the typical features: intractable secretory diarrhoea requiring total parenteral nutrition in all typical cases with congenital onset of symptoms. About 75% of patients died during the first year of life, septicaemia being the cause of death in two-thirds. Cholestasis was an exceptional complication.

Dihydropyrimidinase (5,6-dihydropyrimidine amidohydrolase, DHP, EC 3.5.2.2) is the second enzyme in the degradation pathway of uracil and thymine (see Figure 1). In four patients a deficiency of this enzyme has been suspected because of gross dihydropyrimidinuria and a slightly to moderately increased excretion of uracil and thymine (Duran et al 1990; Henderson et al 1993; Bakkeren et al 1994; Ohba et al 1995).
The enzymatic defect has hitherto been demonstrated in only one patient (Van Gennip et al 1996a,b) because the enzyme is expressed only in hepatocytes. From observations of these four cases, the clinical symptomatology is quite variable, comprising predominantly a neurological disorder.

CASE REPORT

This Turkish boy was born to healthy, consanguineous parents. A 4-year-old brother is in good health and has normal excretion of pyrimidines. Pregnancy was complicated by polyhydramnios and premature labour with spontaneous delivery at 33 + 3 weeks of gestation. The arterial cord blood pH was 7.35; APGAR scores were 5/6/8; body weight was 2950 g.

Immediately after meconium, diarrhoea with green and mucous stools developed. This led to substantial loss of sodium and of weight. Diarrhoea persisted and oral feeding trials failed throughout life. Total parenteral nutrition (TPN) including lipids (1g/kg per day) was started at day 6 and was interrupted for some days because of sepsis during the third week of life. Variable metabolic acidosis persisted (base excess –15.5 to +8mmol/L with alkalosis being rare and not due to buffering with bicarbonate). Icterus neonatorum reached a maximum level of 289 µmol/L (conjugated bilirubin being normal) and was treated by phototherapy. At day 12 bilirubin was 157 µmol/L with 6.8 µmol/L of conjugated bilirubin. At day 13 sepsis with disseminated intravascular coagulation occurred. At day 16 bilirubin was 321 (total)/195 (conjugated) µmol/L. At day 18 bilirubin was 101/58 µmol/L; liver transaminases remained normal (γGT 43U/L). Thereafter cholestasis slowly progressed and liver enzymes began to rise: at 5 weeks ALT was 107U/L, AST 127U/L and AP 811U/L.

Jejunal biopsy at 6 weeks of age revealed congenital microvillous atrophy. Somatostatin therapy did not relieve the gastrointestinal symptoms. Because of the elevated excretion of prostaglandin M (systemic prostaglandin) in urine and thromboxane A2 in stool,
indomethacin was administered for 2 months, also without success. Liver cirrhosis (confirmed by biopsy) had developed at the age of 5 months. The child died at the age of 7 months owing to septicemia. Shortly before death body weight was about 6500g (10th centile) and length and head circumference were slightly below normal. Psychomotor development was about 2 months retarded without specific abnormalities.

METHODS

Uracil and thymine were determined by HPLC following published methods (Simmonds et al 1991; Duran et al 1997). Dihydrouracil and dihydrothymine were analyzed by GC-MS as described by Hoffmann (Hoffmann et al 1989). All four metabolites were also quantified by NMR spectroscopy. $^1$H NMR spectroscopy was carried out on a 600MHz spectrometer at 298K using 60° radiofrequency pulses and 6s repetition time with 132 averages essentially as previously described (Abeling et al 1995; Wevers et al 1995). The pH of the samples was standardized to 2.5 ± 0.10.

The activity of dihydropyrimidinase was determined according to Van Gennip (Van Gennip et al 1997). Briefly, a homogenate (20% w/v) of frozen liver was prepared in a buffer containing 10mmol/L MOPS-NaOH (pH7.4), 1mmol/L EDTA, 10mmol/L dithiothreitol, 5mmol/L 4-(2-aminoethyl)benzenesulfonylfluoride hydrochloride and 10µg/ml leupeptin. After centrifugation, an amount of supernatant corresponding to 0.1–0.2mg of protein was injected into a reaction tube containing 0.1mmol/L Tris-HCl (pH 8.0), 1mmol/L dithiothreitol and 500µmol/L [2-$^{14}$C]dihydrouracil. After 1h incubation at 37°C the reaction was terminated by adding 25µl of 10% (v/v) perchloric acid to the reaction mixture. The $^{14}$CO$_2$ was trapped in a solution of NaOH and determined by liquid scintillation counting. The reaction mixture was centrifuged and an aliquot of the supernatant was used for HPLC. Separation of radiolabelled dihydouracil and its reaction product β-ureidopropionic acid was accomplished by reversed-phase HPLC with UV detection at 205nm and on-line detection of the radioactivity (Van Gennip et al 1997). The activity of dihydropyrimidine dehydrogenase was determined essentially as described (Van Kuilenburg et al 1996). Control values were derived from 8 liver samples.

Prostanoid analyses were performed by gas chromatography–triple-stage quadrupole mass spectrometry (GC-MS-MS) according to previously published methods (Schweer et al 1994).

RESULTS

Echography showed normal anatomy in head, heart and abdomen; ophthalmological examination was normal.

Laboratory investigations showed normal results for TSH, amino acids, creatinine, urea, uric acid (149µmol/L at 5 weeks of age), glucose, lipid electrophoresis (5 weeks), cholesterol, triglycerides, α$_1$-antitrypsin, immunoglobulins and antibodies against toxoplasmosis, syphilis, CMV, hepatitis A, B and C and HIV. Mitochondrial DNA, respiratory chain activity in fibroblasts, cerebrospinal fluid (protein, lactate, cell count), and composition of bile acids and organic acids in urine were normal at the age of 5 weeks. Purine excretion showed normal profiles in urine.
The following abnormal results were obtained:

**Blood:** Na ↓ initially (min. 124 mmol/L); Cl min. 93 mmol/L; pH ↓ frequently (min. 7.25); bilirubin ↑ (see text, 462 µmol/L total/323 µmol/L conjugated before death); lactate 1.4–2.4 mmol/L (normal <2.1); total bile acids 43 µmol/L at 5 weeks (normal 0–6).

Phytosterols were excessively elevated 6 weeks before death, the three most important phytosterols being campesterol (212 µmol/L; normal 0–56), sitosterol (774 µmol/L; normal 0–41) and stigmasterol (177 µmol/L; normal 0). Similar elevations are observed in children receiving TPN with severe liver dysfunction (Clayton et al 1993). Plasma cholesterol was 3.73 mmol/L in this sample.

Several pathological findings relating to haematological parameters, NH₃, trace elements and vitamins were considered to be the consequence of liver dysfunction, hepatosplenomegaly and TPN and are not listed here.

**Stool:** With the exception of K⁺ no reference values are available. Osmolarity 240 mosmol/kg; Na⁺ 99 mmol/L; K⁺ 12 mmol/L (normal about 6); Ca²⁺ 1.3 mmol/L; PO₄³⁻ 2.2 mmol/L; glucose 0.61 mmol/L; creatinine 1.2 mmol/L; urea 9.8 mmol/L; bicarbonate 9.7 mmol/L; reducing substances 0.0–0.5% (on oral feeding of small quantities of watery electrolyte solution, Alfaré and different formula milks). Repeated analyses gave similar results.

Investigation of prostaglandin (PG) metabolite profile gave the following results: PGE₂ 79.9 pg/ml; 6-keto-PGF₁α not detectable; thromboxane B₂ (Tx B₂) 374 pg/ml (direct correlation to thromboxane A₂); PGE-M 60 pg/ml; 2,3-dinor-6-keto-PGF₁α 3.8 pg/ml; 2,3-dinor-TxB₂ not detectable; 11-dehydro-TxB₂ 37.1 pg/ml. Normal values for stool do not exist; compared to urine profiles and to some other stool analyses, thromboxane concentration seems to be very high, particularly because the ratio of thromboxane to PGE₂, normally is inverse (see normal values for urine below).

**Urine:** Osmolarity 41–185 mosmol/kg (normal 570–1300); Na⁺ 0.7–5.4 mmol/L (<20 is indicative of Na deficiency); K⁺ 0.6–7.8 mmol/L (normal >10); Ca²⁺ 0.3–1.67 mmol/mmol creatinine (normal <0.5); creatinine 628–1688 µmol/L; frequently slight proteinuria with mainly α₁-microglobulins; albumin 0.003–0.007 mg/L; variable slight elevations of l-lactate and pyruvate with normal ratios.

**Urinary prostaglandin metabolites** (excretion expressed as ng/h per 1.73 m²; normal ranges from the 10th to the 90th centile given in brackets) were as follows: **Renal prostaglandins:** PGE₂ 91.3 pg/ml (6.4 ng/h per 1.73 m²; normal 4–27); 6-keto-PGF₁α 152 pg/ml (10.6 ng/h per 1.73 m²; normal 78–23); Tx B₂ 55 pg/ml (3.9 ng/h per 1.73 m²; normal 1–21). **Systemic prostaglandins:** PGE-M 28900 pg/ml (2022 ng/h per 1.73 m²; normal 110–1140); 2,3-dinor-6-keto-PGF₁α 68.4 pg/ml (4.8 ng/h per 1.73 m²; normal 4–19); 2,3-dinor-TxB₂ 286 pg/ml (20.0 ng/h per 1.73 m²; normal 8–36); 11-dehydro-TxB₂ 854 pg/ml (59.8 ng/h per 1.73 m²; normal 15–87). Thus, renal prostaglandin synthesis was normal and systemic PGE-M production was elevated.

**Urinary pyrimidines:** Initial GC-MS investigation of organic acids in urine showed huge increases of dihydrouracil and dihydrothymine. Specific HPLC analysis of purines and pyrimidines revealed slightly elevated uracil and thymine concentrations also (see Table 1 and Figure 2). Otherwise the profile of excreted purines was normal.
Cerebrospinal fluid: Uracil not detectable; thymine elevated; dihydrouracil and dihydrothymine strongly elevated (see Table 1 and Figure 2).

Gut biopsy: Biopsy of proximal jejunum showed congenital microvillous atrophy with atrophy of villi and inclusions of microvilli and granules in enterocytes (‘inclusion disease’).

Liver biopsy: Liver biopsy showed biliary cirrhosis; activity of DHP < 3 nmol/h per mg protein (controls 20 – 82; \( n = 8 \)); activity of dihydropyrimidine dehydrogenase (EC 1.3.1.2; DPD) 21 nmol/h per mg protein (controls 5 – 14; \( n = 8 \)).

DISCUSSION

Our patient suffered from congenital microvillous atrophy (McKusick 251850), a recessive disorder first described in 1978 by Davidson and colleagues. Its pathogenesis remains uncertain. A survey of 23 cases (Phillips and Schmitz 1992) delineates the typical features. As in our patient, histological examination reveals villous atrophy as well as PAS-positive ‘secretory’ granules in the apical cytoplasm of enterocytes and inclusions of microvilli in the cytoplasm (‘inclusion disease’). The vast majority of patients had a congenital form of microvillous atrophy with onset of diarrhoea within the first week of life (Phillips and Schmitz 1992). In those patients the PAS staining abnormality appears in the upper crypt epithelium, whereas in the late-onset form the histological abnormality is found in the low villous epithelium. There is also an ‘atypical form’ with the histological abnormalities in the low crypt region and onset of diarrhoea either congenital or later (Phillips and Schmitz 1992).

Besides intractable secretory diarrhoea our patient also showed intermittent proteinuria with predominance of \( \alpha_1 \)-microglobulin and very low albumin excretion. Bindl and colleagues (1996) demonstrated a characteristic pattern of proteinuria in 4 patients with proven microvillous atrophy (our patient included), and speculated that this might be the renal manifestation of microvillous atrophy concerning transmembraneous protein transport by pinocytosis.

However, in addition to the typical features of congenital microvillous atrophy, our

Table 1  Concentrations of pyrimidines in urine relative to creatinine and in CSF in the index patient with dihydropyrimidinase deficiency

<table>
<thead>
<tr>
<th></th>
<th>Uracil (mmol/mol creatinine)</th>
<th>Thymine (mmol/mol creatinine)</th>
<th>Dihydrouracil (µmol/L)</th>
<th>Dihydrothymine (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>26 – 93</td>
<td>7 – 40</td>
<td>436 – 662</td>
<td>274 – 585</td>
</tr>
<tr>
<td>CSF (µmol/L)</td>
<td>nd</td>
<td>3</td>
<td>117</td>
<td>179</td>
</tr>
</tbody>
</table>

Urinary levels were determined by HPLC (uracil and thymine) and GC-MS (dihydrouracil and dihydrothymine). One urine was analysed by NMR spectroscopy with comparable results (see Figure 2); CSF values are only available from a single determination performed by NMR spectroscopy (see Figure 2).

References:
- Assmann and Haas (1990); Van Gennip et al (1994)
patient differed in several aspects from the other patients described: all 23 children were
born between 35 and 40 weeks of gestation and none of 19 patients where this information
was available had presented with polyhydramnios. Furthermore, our patient developed
severe cholestasis by 2 weeks of age, leading to liver cirrhosis. Only one of the other
patients described had developed cholestasis without cirrhosis (time of onset is not given).

In our patient a primary defect of bile acid biosynthesis could be excluded. The role of
phytosterols (derived from intralipid infusions) in the development of cholestasis has not
yet been completely elucidated (Clayton et al 1993), but 6 days of intralipid are not
sufficient to cause cholestasis.

Variable lactic acidosis and high renal excretion of systemic prostaglandins are
interpreted as the result of imbalances in electrolyte and fluid homeostasis. The apparently
very high excretion of thromboxane in stool is suspected to be due to chronic inflammation.

Dihydropyrimidinase deficiency (McKusick 222748) is characterized by a grossly
elevated excretion of dihydouracil and dihydrothymine as well as moderately or slightly
increased excretion of uracil and thymine. With classical chromatographic methods
(HPLC, GC-MS) reliable simultaneous identification and quantification of all four
metabolites is difficult, but this can be achieved by NMR spectroscopy (see Figure 2).
Using HPLC the pyrimidine bases can be analysed reliably (Van Gennip et al 1981; Assmann and Haas 1990). The dihydropyrimidines can be analysed by amino acid analyser after isolation and conversion into the corresponding β-amino acids (Van Gennip et al 1993) or by quantitative organic acid analysis (Hoffmann et al 1989).

With NMR spectroscopy the relevant compounds for making the diagnosis can be demonstrated both in urine and in CSF (see Figure 2). The same holds for plasma or serum (data not shown). Furthermore, the absence of detectable amounts of N-carbamyl-β-alanine in the NMR spectrum excluded ureidopropionase deficiency as a possible defect in this patient and suggested dihydropyrimidinase deficiency as the likely defect, which was later confirmed enzymatically. In this way NMR spectroscopy may help in pinpointing an enzyme defect.

In our patient the elevated excretion of pyrimidines was found to be the consequence of a primary defect of dihydropyrimidinase (DHP) in the liver. DHP activity is not detectable in red and white blood cells or in fibroblasts. In contrast, we found normal excretion of pyrimidines in two other patients with confirmed microvillous atrophy (data not shown). There are so far 4 patients known with suspected DHP deficiency (Duran et al 1990; Henderson et al 1993; Bakkeren et al 1994; Ohba et al 1995). In only one of them has the enzyme deficiency been demonstrated because of the necessity for liver biopsy (Van Gennip et al 1996, 1997). The clinical course of isolated dihydropyrimidinase deficiency is variable but seems to focus on neurological symptoms similar to patients with dihydropyrimidine dehydrogenase deficiency. This may be caused by a decrease of β-alanine due to deficient pyrimidine catabolism (deficiency of either the first or the second enzyme of the pathway leading to β-alanine, see Figure 1). β-Alanine is suspected to have a function as neurotransmitter but this is not yet clearly defined (De Feudis and Del Rio 1977; Scriver and Gibson 1995). Possible toxic effects of the increased metabolites also have to be considered. The known neurotoxicity of fluorouracil in patients with a defect of dihydropyrimidine dehydrogenase seems to be the consequence of elevated fluorouracil levels.

In our patient the severe clinical course was determined by gastrointestinal and liver disease leading to death in infancy. It remains speculative whether he suffered from two independently inherited recessive metabolic diseases or if he had a hitherto undescribed contiguous gene syndrome due to a chromosomal microdeletion/microduplication. To date, the chromosomal locations of both dihydropyrimidinase deficiency and microvillous atrophy are unknown.

REFERENCES


