Case Report

Successful intrauterine treatment of a patient with cobalamin C defect

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A B S T R A C T
Cobalamin C (cblC) defect is an inherited autosomal recessive disorder that affects cobalamin metabolism. Patients are treated with hydroxycobalamin to ameliorate the clinical features of early-onset disease and prevent clinical symptoms in late-onset disease. Here we describe a patient in whom prenatal maternal treatment with 30 mg/week hydroxycobalamin and 5 mg/day folic acid from week 15 of pregnancy prevented disease manifestation in a girl who is now 11 years old with normal IQ and only mild ophthalmic findings. The affected older sister received postnatal treatment only and is severely intellectually disabled with severe ophthalmic symptoms. This case highlights the potential of early, high-dose intrauterine treatment in a fetus affected by the cblC defect.

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1. Introduction

Cobalamin C (cblC) defect is the most common disorder that affects intracellular cobalamin metabolism. The conversion of dietary cobalamin into its two metabolically active forms, adenosylcobalamin and methylcobalamin is impaired, resulting in elevated homocysteine (Hcy) and methylmalonic acid concentrations in body fluids [1]. A number of mutations in the MMACHC gene have been characterised resulting in symptomatic cblC defect, which is inherited in an autosomal recessive manner [2]. Clinical features comprise failure to thrive, mental retardation, visual impairment, hydrocephalus and congenital cardiac anomalies. Early treatment with hydroxycobalamin (OHCbl), betaine and folate has a positive impact on survival and severe organ damage. However, visual impairment and neurocognitive disability can rarely be prevented in early-onset disease [1]. Here we report a favourable outcome in the younger of two affected female siblings following prenatal treatment with 30 mg/week OHCbl from week 15 of pregnancy until delivery.

Abbreviations: cblC, cobalamin C; Hcy, homocysteine; OHCbl, hydroxycobalamin; tHcy, total homocysteine; McBl, methylcobalamin; AdoCbl, adenosylcobalamin; CNCbl, cyanocobalamin.

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2. Patients and methods

Biochemical and enzymatic prenatal diagnosis was performed at 15 weeks of gestation during the second pregnancy of a woman who had two years previously given birth to a female child (sibling 1) with severe early-onset cblC defect. This child exhibits multiple developmental problems despite treatment with OHCbl, betaine and folic acid from week 4 of life [4]. She is now 16 years of age, unable to walk and to speak, and has severe visual impairment and behavioural problems. The parents are of Caucasian origin, no consanguinity. She has one older non-affected sibling.

Following confirmation of cblC defect in the fetus during the second pregnancy, the mother was treated from week 15 of gestation with intramuscular OHCbl (3 × 10 mg/week) and folic acid (5 mg/day) after confirmation that this treatment was an individual treatment attempt. Postnatal treatment of sibling 2 consisted of oral betaine 200 mg/kg BW/day, folic acid 5 mg/day and intramuscular OHCbl 1 mg/day. At last follow-up at the age of 11 years, the child was treated with 2 × 10 mg intramuscular OHCbl per week, oral betaine 180 mg/kg/day and folic acid 5 mg/day (Table 1c).

Biochemical analyses of serum total Hcy (tHcy), propionylcarnitine and methylmalonic acid in urine were performed by tandem MS and HPLC/MS/MS respectively (Zentrum für Stoffwechseldiagnostik Reutlingen GmbH, Reutlingen, Germany). Enzymatic assessment and mutational analysis of the MMACHC gene were performed as described elsewhere [3] (Fig. 1).
Table 1

| a) Biochemical prenatal (15th week of gestation) and postnatal findings | Sibling 2 | Normal |
|---|---|---|
| Propionylcarnitine in amniotic fluid (13 weeks of gestation) | 3.8 μmol/l | 0.8-1.8 μmol/l (n = 5) |
| Methylmalonic acid in amniotic fluid | 11.7 μmol/l | <0.7 μmol/l (n = 5) |
| Vit B12 in serum under treatment (mother) | >20,000 pg/ml | 160-1100 pg/ml |
| Homocysteine in cord blood | 45.1 μmol/l | <8 μmol/l |
| Homocysteine in serum at day 1 | 27.3 μmol/l | <12 μmol/l |
| Vit B12 in serum at day 1 | >20,000 pg/ml | 160-1100 pg/ml |
| Homocysteine in serum day 7 | 41.7 μmol/l | <12 μmol/l |
| Propionylcarnitine in dried blood at day 7 | 5 μmol/l | <6.8 μmol/l |
| Methylmalonic acid excretion in urine at day 7 | 66 mmol/mol creatinine | 0-10 mmol/mol creatinine |

| b) Biochemical findings postnatal | Sibling 1 | Sibling 2 | Normal |
|---|---|---|---|
| Methylmalonic acid in urine at 20 days of age | 1940 mmol/mol creatinine | 60 mmol/mol creatinine | 0-10 mmol/mol creatinine |
| Methylmalonic acid in urine at 20 days/at day 1 | 32 | 5 | 6-8 |
| Homocysteine in serum at 20 days of age | 282 μmol/l | 41 | <8 |
| Actual methylmalonic acid in plasma | 9.1 μmol/l | 2.7 μmol/l | <0.26 μmol/l |
| Actual homocysteine serum | Not available | 72 μmol/l | <8 |
| Actual methionine in serum | 37 μmol/l | 23 μmol/l | 15-45 μmol/l |
| Actual methylmalonic acid in urine | Not available | 17.6 mmol/mol creatinine | 0-10 mmol/mol creatinine |

| Clinical findings | Sibling 1 | Sibling 2 |
|---|---|---|
| Weight at birth | 2570 (1st perc.) | 3100 (10th perc.) |
| Circumference at birth | 33(1st perc.) | 35 (25th perc.) |
| General findings at infancy at birth | Hypotonia, feeding problems, heart murmur at day 20, myoclonic seizures, horizontal nystagmus, respiratory distress | Normal clinical findings |
| MRI of brain at day 29/day 7 | Diffuse supratentorial cortical atrophy without signs of hypoxic-ischaemic lesions | Slightly delayed white matter maturation and asymmetric increased signal intensities of the white layer |
| Actual developmental test, IQ | Denver test corresponds to 8-11 months of age | WISC-IV total IQ 103 |
| Actual somatic status | 32 kg (z-score –5), height ca. 140 cm (<3rd perc.), HC 48 cm (<3rd perc.) | Height 150 cm (47th perc.), weight 34 kg (21st perc.), HC 52.5 (9th perc.) |
| Ophthalmic findings at 3 months of age | Bil loss of photoreceptors in the central region of the macula and partial optic atrophy | Not investigated |
| Actual ophthalmic findings | Bil nystagmus, bil disc pallor, fundi? (no cooperation) | Acuity (distance) 20/30, no nystagmus, minor marginal disc pallor, normal fundi |

| c) Actual treatment | Hydroxycoelamin | 2 x 10 mg. I.M.,week |
|---|---|---|
| Follic acid | 5 mg/day | 2 x 10 mg. I.M.,week |
| Betaine | 180 mg/kg BW/day | 5 mg/day |
| Piperperone | 3 x 20 mg. p.o. | 180 mg/kg BW/day |
| Atomoxetine | 1 x 18 mg. p.o. | |

* Actual: sibling 1: 16 years, sibling 2: 11 years of age.

3. Results

Biochemical and clinical findings are summarised in Table 1a–c. No complications during pregnancy and delivery of the female sibling 2 following intrauterine treatment were reported and the infant appeared to be healthy and without congenital anomalies. MRI of the brain after birth showed slightly delayed white matter maturation and asymmetric increased signal intensities of the white layer (Table 1b). In contrast, sibling 1 showed diffuse supratentorial cortical atrophy without signs of hypoxic-ischaemic lesions and eye findings revealed bilateral loss of photoreceptors in the central region of the macula and partial optic atrophy at 29 days of age (Table 1b). Data of the very different psychomotor development in both siblings are shown in Table 1b. Sibling 2 attends secondary school with excellent success and has a total IQ of 103 (WISC-IV). The affected older sibling shows in the Denver test a corresponding development of an 8–11 months infant even treated from 4 weeks of age with OHCB, betaine and folic acid as described [4]. For details we refer to reference [4]. She is unable to walk, lacks speech development and shows an aggressive behaviour which is partly controlled by pipamperon and atomoxetine (Table 1c).

Biochemical results from amniotic fluid at gestational week 15 as well as postnatal serum and urine for sibling 2 are summarised in Table 1a. Values for methylmalonic acid and homocysteine were prenatally and post-natally elevated. However, methylmalonic acid excretion over the years remained below 60 mmol/mol creatinine (normal, <20 mmol/mol) and serum tHcy between 40 and 60 μmol/l (reference, <12 μmol/l).

Both affected children share the same genotype and similar residual enzymatic activity (Table 2). Total uptake of [14C]erythronylacacetate was strongly reduced compared to values of 32 control individuals (Table 2). The percentage of methylcobalamin (MeCbl) and adenosylcobalamin (AdoCbl) was reduced in comparison to the mean control value, the percentage of cyanocobalamin (CNbCbl) was increased and the percentage of OHbCbl was in range of the control values for both children (Table 2).

Monitoring of serum tHcy over time shows values <100 μmol/l in both siblings (in sibling 1 from 3 months of age, Fig. 2). However, variations in tHcy were generally higher in the girl without prenatal OHbCbl treatment.

Methylmalonic acid in urine was only slightly elevated in both siblings (Table 1). Decrease of methylmalonic excretion in urine and methionine concentration in serum of sibling 1 is shown in Fig. 3 indicating rapid response to the treatment in sibling 1.

4. Discussion

The high rate of congenital anomalies, including heart defects and CNS involvement, observed in early-onset cblC defect patients suggests that enzymatic dysfunction during embryonal and/or fetal stages of development contribute to the clinical phenotype [1] and that postnatal treatment alone may not be sufficient to support normal psychomotor development and prevent ocular damage [5, 6]. The outcome in our prenatally treated patient reported here with cblC defect was much more favourable than in a case previously described [7], in which prenatal treatment was initiated at a lower dose and at a later gestational stage (2 mg/week OHbCbl from week 25 compared to 30 mg/week OHbCbl from week 15 in our patient). In the case described by Hupperer et al. [7] retinopathy and cognitive impairment could not be prevented and tHcy was highly elevated (160.9 μmol/l) in a neonate despite prenatal treatment. Methylmalonic acid excretion was also significantly elevated (393 mmol/mol creatinine). In our patient, tHcy was 27.3 μmol/l and methylmalonic acid excretion was 66 mmol/mol creatinine after delivery, suggesting a better therapeutic effect associated with the higher dosage of OHbCbl on the fetus (Table 1a).

Both the low level of enzymatic activity and the MMACHC locus genotype (c.457C>T, p.Arg153*/c.271dupA, p.Arg91Lysfs*14) suggest a
severe congenital defect in the siblings reported here. The same genotype has been described in several patients with early-onset cblC defect [2, 8, 9] and no patients with this genotype and late-onset disorder have been described. In addition, the nonsense mutation (c.457C>T, p.Arg153*) has been described in combination with c.331C>T (p.Arg111*) in a further early-onset patient [2] and in combination with a missense mutation (c.365A>G, p.His122Arg) in patients with late-onset disease [10].

The contribution of additional factors beyond prenatal treatment that may have influenced the contrasting outcomes in the two siblings described here cannot be excluded. A large variation in clinical symptoms between siblings with late-onset disease is well known [11–13]. Augoustides-Savvopoulou et al. describe a child with early-onset symptoms of cblC defect with severe neonatal seizures, developmental delay and spastic paraparesis who died at the age of 13 years, while the child’s

Table 2
Comparison of intracellular metabolic processing of CNCbl (fibroblasts) in siblings with identical genotype compared to control individuals (n = 32). AdoCbl, adenosylcobalamin; CNCbl, cyanocobalamin; MeCbl, methylcobalamin; OHCbl, hydroxycobalamin.

|                     | Patient ID 160 | Patient ID 959 | Control value mean (range) |
|---------------------|----------------|----------------|---------------------------|
| [57Co]cyanocobalamin uptake (pg/mg protein) |                     |                |                           |
| Total uptake        | 8.8            | 10             | 71 (19–142)               |
| Cobalamin coenzyme synthesis from [57Co]cyanocobalamin (distribution of cobalamins: % of total cobalamins) |                     |                |                           |
| MeCbl               | 3.3            | 1.0            | 60 (48–78)                |
| AdoCbl              | 3.8            | 3.5            | 14 (6.9–30)               |
| CNCbl               | 82             | 77             | 8.7 (3.3–18)              |
| OHCbl               | 10             | 18             | 16 (7.3–25)               |

Fig. 1. A) Normally developed sibling 2 at the age of seven years (prenatal and postnatal treatment); B) severely intellectually disabled sibling 1 at the age of 12 years (postnatal treatment only).

Fig. 2. Serum homocysteine concentration in two siblings with cblC defect from day 1 in the antenatally-treated patient (sibling 2) and in her older sister (sibling 1) who was detected by selective screening of organic acids in urine on day 29 [Table 2 and citation [4]].
sibling developed apparently normally until the age of ten years, when a
decline in cognitive abilities, ataxia and myoclonic jerks occurred [14].
Also our case (sibling 1) suffered from metabolic decompensation at
3 weeks of age which may have contributed to the unfavourable
development. However, eye and cerebral MRI findings of the brain indicate
that these early changes may have developed prenatally and are not
likely to be caused by increased homocysteine levels in the first
3 weeks of life or by metabolic decompensation. The very rapid decrease
of methylmalonic excretion in urine and normalization of plasma me-
thionine indicate the otherwise excellent reaction on OHCbl treatment
(Fig. 3). There were no different treatment modalities in both children,
however, there is some uncertainty about a “safe” level of homocysteine in
respect to an impact on the neurologic development. There are no
long term prospective studies from birth showing the influence of ele-
vated homocysteine on the psychomotoric/ocular development [6].

In conclusion, this very limited experience suggests that early prena-
tal treatment may prevent disease manifestations in patients at risk of
developing a cblC defect phenotype. With successful newborn screening
the possibility of improving outcome in patients with cblC defect has al-
ready been established [5, 15, 16], however prenatal diagnosis and
treatment together with lifelong postnatal treatment may represent
an opportunity to prevent the manifestation of clinical symptoms alto-
gether. Our results suggest that intrauterine treatment should be initiat-
ed as early as possible in an affected fetus and continued throughout
gestation. The high dosage used here resulted in a high Vit B12 concen-
tration in the mother’s as well as in the fetal blood circulation as docu-
mented at day 1 of life. The importance of high OHCbl dosages to
reduce homocysteine and methylmalonic acid have been demonstrated
in a 13 year old patient [17]. Further experiences in similar cases during
pregnancy should be collected in the future and Vit B12 and postnatal
homocysteine levels should be monitored closely in affected patients
to better understand the impact of OHCbl dosage on homocysteine
levels in serum [18].

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