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Betaine in the treatment of homocystinuria due to 5,10-methylenetetrahydrofolate reductase deficiency*

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Abstract. In a 3-year-old mentally retarded girl with homocystinuria due to 5,10-methylenetetrahydrofolate reductase deficiency among different therapeutic approaches only treatment with betaine (15–20 g/day) resulted in a satisfactory biochemical response. Betaine improved homocysteine remethylation and thus lowered plasma homocystine to trace amounts and normalized the previously very low plasma methionine concentration. This biochemical response was associated with a clinical improvement although she remained mentally retarded.

Key words: Homocystinuria – 5,10-methylenetetrahydrofolate reductase deficiency – Betaine – Homocysteine remethylation

Introduction

In 5,10-methylenetetrahydrofolate reductase (MTHFR) deficiency homocysteine remethylation to methionine is impaired due to lack of the endogenously formed methyl donor, 5-methyltetrahydrofolate (5-MTHF). As a consequence patients show homocystinuria and homocystinemia of moderate degree and decreased plasma and tissue concentrations of methionine. All patients, about 20, diagnosed up to now [for references see 2, 14] showed neurological dysfunction of variable type and severity, eventually manifesting as progressive neonatal leukoencephalomyopathy [1].

The etiology of the neurological dysfunction remains unclear. Reduced capacity of methionine biosynthesis in the patients most accurately reflects the clinical severity [2] and may be of major pathogenetic importance, causing substrate deficiency for S-adenosyl-L-methionine (SAMe) formation. SAMe is the methyl donor for many methyl transfer reactions in the body, being involved in neurotransmitter, carnitine, phosphatidylcholine, and subsequently also in myelin synthesis. Cerebral thromboembolism being related to homocysteine accumulation or deficient brain folates may also be involved in the neurological damage.

In most cases no effective therapy has been found. Two patients responded to folates [6], another two patients with onset of the disease in early infancy responded to therapy with methionine, vitamins B_6 and B_{12} and folinic acid [8] or folates, methionine and carnitine [1], respectively.

In our patient these measures did not lead to biochemical improvement. However, it was possible to improve homocysteine remethylation by stimulating the betaine-dependent pathway with oral supplementation of betaine monohydrate.

Case report

C.M., the second child of a nonconsanguineous Greek couple, came to our attention at 2 years of age because of marked psychomotor retardation. Gestation and delivery were normal (birth weight 3250 g, length 52 cm, head circumference 33 cm). According to her mother she had been doing well until the age of 5 months when psychomotor retardation became obvious. From months 4–6 she was hospitalized because of congenital subluxation of the hip.

At 2 years of age she was microcephalic (head circumference: 43 cm). Internal and restless, showed athetoid movements of her arms and myotonic jerks of the lower extremities. She could not sit without support. Tendon reflexes were easily obtainable. She grasped objects and put them into her mouth. Social contact was poor. Drooling, grimacing and stereotype smacking movements of the mouth were observed. She could not speak, however, periodically she screamed without motive. Using the Denver Developmental Screening Test, she was found to function at a level of 6 months.

Routine laboratory evaluations, including cerebrospinal fluid analysis, were unremarkable. A CAT scan revealed mild internal and external hydrocephalus. EEG showed some dysrhythmia, seizures were never observed. Nerve conduction velocity was normal as were ophthalmological examinations.

She had mild homocystinuria and cystathioninuria. Urinary homocystine excretion was 20–40 µmol/day, that of cystathionine was twice as much. The plasma concentrations of the relevant amino acids were: homocystine: 15–25 µmol/l, mixed homocysteine-cysteine disulfide: 25–35 µmol/l, cystine: 25 µmol/l, cystathionine: traces, and methionine: 4–5 µmol/l. Methylmalonic acid was not detectable by gas chromatography. Serum folate (3 ng/ml) and free carnitine concentration (18.4 µmol/l) were low, cobalamin was normal.

An oral methionine loading test of 100 mg L-methionine per kg body weight [3, 13] showed a normal disappearance of methionine from the plasma.

5,10-methylenetetrahydrofolate reductase activity measured in extracts of lymphocytes and cultured skin fibroblasts was less than 2% of control values. Detailed data have been published [17].

Therapy was started at the age of $2\frac{1}{4}$ years. The different regimens are shown in Table 1 and were changed every 4–6 weeks.

Family history. The first-born sister was microcephalic and severely mentally retarded. She had an almost identical course of disease. Since the age of $1\frac{1}{2}$ years she had regressed rapidly and had three episodes of deep coma and respiratory failure. At $2\frac{1}{2}$ years she died in coma. No diagnosis had been established. Postmortem examination revealed multiple thromboses in various

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Table 1. Plasma amino acid concentrations before and during therapy

Therapy	Methionine	Half cystine (μmol/l)	Homocystine
(1) None	4–5	25	15–25
(2) Vitamin B_6 (240 mg/day)	4	25	23
(3) Folic acid (20 mg/day)	5	24	24
(4) Folic acid (20 mg/day) Methionine (1 g/day)	10–89[a]	48–60	14–19
(4) Folic acid (80 mg/day) Methionine (1 g/day)	10–28[a]	45	12–17
(4) Folinic acid (60 mg/day) Methionine (1 g/day)	21–115[a]	45–66	12–18
(4) Folinic acid (60 mg/day) Vitamin B_6 (240 mg/day) Methionine (1 g/day)	8–112[a]	35–70	11–15
(5) Betaine (6 g/day) Folinic acid (15 mg/day)	12–19	58–69	3
(6) Betaine (12 g/day) Folinic acid (15 mg/day)	19–22	55	Trace
(7) Betaine (15 g/day) Folinic acid (15 mg/day)	30	55	Trace
(8) Betaine (20 g/day) Folinic acid (15 mg/day)	23–45	65–90	Trace

Body weight was 12.5–13.5 kg
Permanent treatment with antiplatelet drugs (dipyridamole 100 mg/day and acetyl-salicylic acid 375 mg/day) until administration of 15 g betaine/day
[a] Wide fluctuations at different intervals after methionine intake.
Traces of cystathionine were always present. The concentration of the mixed homocysteine-cysteine disulfide remained rather constant (18–42 μmol/l), also when on betaine

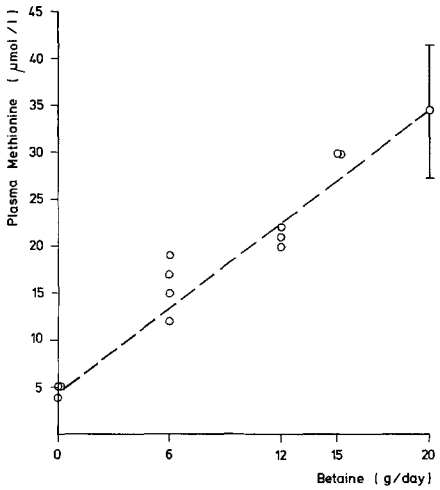

Fig. 1. Methionine concentration in plasma as a function of the intake of betaine monohydrate. Methionine concentrations were measured every 4–6 weeks after raising the betaine dosage. The values represent fasting plasma concentrations. Reaching 20 g betaine/day plasma samples were taken monthly at variable intervals to betaine intake. The values represent the mean ± S.D. of 14 plasma samples

and small cerebral vessels[1]. Widespread necroses in the cerebral cortex and medulla and areas of demyelination in the brainstem and spinal cord out of proportion to the vascular changes had been found. These findings are compatible with homocystinuria due to MTHFR-deficiency. Identical findings in a patient with MTHFR deficiency had been reported by Kanwar et al. [9].

Intermediate levels of MTHFR-activity were observed in the parents and in one brother prenatally and after birth [17].

Laboratory methods. Plasma samples were obtained from heparinized venous blood by immediate centrifugation. The precipitation of proteins with 5% sulfosalicylic acid was directly performed thereafter. The supernatant obtained after precipitation was stored at −20° C until analysis. Urine was collected on ice and kept deep-frozen until analysis. Quantitative amino acid analyses were performed on an LKB 4400 amino acid analyzer using lithium citrate buffers and standard programs.

1 Neuropathological examinations were done by Prof. J. Pfeiffer, Institut für Hirnforschung, and Prof. W. Schlote, Patholo-

Results

The child was on various therapeutic regimens for 18 months. The effects on the sulfur-containing free plasma amino acids are shown on Table 1. There was no significant response to vitamin B_6 and folates, given alone or in combination. A methionine supplement of 1 g/day, given in four doses, resulted in a high but unstable rise of the plasma methionine levels, fluctuating according to the methionine intake. The concentrations homocysteine disulfide remained mainly unchanged, while the plasma cystine concentration was normalized.

A marked improvement was only noted after the administration of betaine monohydrate (6 g/day): plasma homocystine dropped to trace amounts. The stepwise increase of betaine, every 4–6 weeks, up to 20 g/day, resulted in a dose-related rise of plasma methionine into the normal range (Fig. 1). The methionine levels were stable over the day without wide oscillations. Though homocystine disappeared from blood the level of the mixed cysteine-homocysteine disulfide remained constant. Cystine concentration was normal. Serine and glycine, decomposition products of betaine, did not accumulate in plasma.

The urinary output of homocystine and cystathionine varied considerably during all therapeutic regimens. Although urinary homocystine decreased when the child was on betaine it was nevertheless still excreted.

During betaine therapy the child's motor function improved. She became more alert and interested in her surroundings and responded to her mother. She learned to crawl to objects of interest, to stand alone with a little support and to walk with support. The muscular tone improved. Still, she remained severely mentally retarded and could not speak, however, she stopped grimacing

increments of somatic and skull growth became normal. At the age of 4 years length was 96 cm, weight 13.2 kg, and head circumference 55.0 cm.

During therapy total serum folates were high (>100 ng/ml), serum cobalamin remained normal without supplements and free serum carnitine remained low (11 μmol/l). For more than half a year the maximum dose of 20 g betaine monohydrate per day was administered to the girl now weighing 13 kg without apparent harmful effects and without signs of a disturbed liver function.

Discussion

Using a regimen, comprising vitamin B_6, folates and methionine, as proposed by Harpey et al. [8] our patient did not show a satisfactory response, however, we achieved good biochemical control by high-dose betaine supplement. Administration of as much as 20 g betaine monohydrate per day led to an increase and normalization of the plasma methionine concentration with only minor fluctuations and to a reduction of plasma homocystine to trace levels. Associated with the use of betaine the child improved clinically, however, still remaining mentally retarded.

Recycling of homocysteine is necessary for maintaining intracellular methionine levels [12]. Two pathways exist for homocysteine remethylation to methionine [11]. While the folate-dependent pathway (5-MTHF-homocysteine methyltransferase—EC 2.1.1.13), being ubiquitously distributed and being the more important one in humans, is impaired in MTHFR deficiency, the flux through the betaine-dependent pathway (betaine-homocysteine methyltransferase—EC 2.1.1.5) can apparently be enhanced by supplementation of the methyl donor betaine.

Low betaine doses have been shown to have no biochemical effect [3, 10]. High doses, however, proved to be effective in stimulating the betaine dependent methylating pathway: patients with homocystinuria due to cystathionine-β-synthetase deficiency, when on 6–10 g betaine per day, showed a substantial reduction in plasma homocystine concentrations combined with a further increase in the already highly elevated methionine blood levels and sometimes a striking clinical improvement [16, 18]. Obviously there is some variability in methionine response to betaine in different patients.

In disorders of homocysteine remethionine is acting against two biochemical disturbances: homocystinemia and hypomethioninemia. Moderate degrees of homocystinemia bear the risk of inducing fatal thromboembolic complications in the brain [9] as was demonstrated here by the autopsy of the patient's sister. They should therefore be prevented.

Methionine deficiency of the brain with secondary reduction of neurotransmitter and myelin synthesis might be balanced more adequately by betaine treatment than by methionine supplementation. Using betaine, the tissues containing betaine-homocysteine methyltransferase activity, such as liver, kidney and brain [7], could meet their methionine requirements by in situ synthesis and in addition by uptake from the blood [19], after normalization of blood methionine concentrations.

The persisting homocystinuria and the constantly elevated levels of cysteine-homocysteine disulfide in blood and urine, indicate a continous production of homocysteine in the body during betaine treatment. Total correction of the homocysteine-methionine remethylation by betaine could not be expected since the betaine methylating enzyme is not present in every organ. Replenishment of methionine in tissues when the patient is on betaine [5, 7] increases the methionine metabolism and in consequence leads to enhanced production of homocysteine and to increased plasma cystine levels. The still reduced urinary excretion of homocystine, however, denotes a highly stimulated flux of homocysteine through the transmethylation pathway.

Recently betaine was added to vitamin B_{12} treatment in a child with homocystinuria due to an abnormal cobalamin metabolism [18], causing some biochemical response.

The relationship of the deficient brain folates to the neurological damage remains speculative. Although 5-MTHF is the fraction of folate in blood and tissues its only function is to participate in methionine biosynthesis [4, 15].

Cystathioninuria in this patient, not being influenced by vitamin B_6, was the result of an unbalanced formation and utilization of cystathionine caused by an increased homocysteine concentration. Cystathioninuria was reported only once in another patient with a disorder of homocysteine remethylation.

MTHFR deficiency has turned out to be heterogenous with respect to the degree of neurological dysfunction, residual enzyme activity probably enzyme methionine biosynthesis, and response to therapy. In patients with unresponsiveness to folates it seems to be reasonable to try betaine in high doses as early as possible.

For further treatment of this patient a permanent supplement of 12 g daily of betaine would be sufficient to obtain a reasonable plasma methionine level with only a trace of homocystine and a normal cystine content. Continous folinic acid treatment has not so far been proven effective.

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After another six months of betaine treatment (15 g betaine/day) the child's motor function had persistently improved and she is now able to walk without support.

Recently we startet betaine treatment (15 g betaine-monohydrate/day) in a 15-month-old severely mentally retarded child with MTHFR-deficiency. Plasma homocystine dropped immediately from 40 µmol/l to traces and plasma methionine increased from 14 to 34 µmol/l. One month of treatment resulted in a surprisingly profound improvement of the child's psychomotor function.

Letters to the editor

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Fatal infantile cardiac glycogenosis without acid maltase deficiency presenting as congenital hydrops

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Sir,—We discuss here an unusual case of congenital hydrops with cardiomyopathy. The infant, born at 37 weeks gestation, was a grossly hydropic male with ascites, enlarged tongue, and heart murmur. Workup for immune hydrops was negative. The electrocardiogram was abnormal, showing a Wolf-Parkinson-White pattern. Considerable cardiomegaly was found radiologically. The heart condition worsened and the infant succumbed due to cardiorespiratory arrest on day 18. Autopsy performed 16 h after death revealed an enlarged and hypertrophic heart. The weight was 80 g (normal 23 ± 14 g). Thickness of the right ventricular wall was 1.2 cm (normal 0.4–0.6). Thickness of the left ventricular wall was 1.5 cm (normal 0.3–0.7). Prominent glycogen deposits were found in the heart myocardium and, to a lesser extent, in the skeletal muscle (light microscopy). Although postmortem autolysis made it impossible to identify clearly membrane-bound glycogen deposits, fragments of lysosomal membranes were found adjacent to glycogen deposits in the heart (electron microscopy). The glycogen content of the heart was 7.8% (normal less than 1.8%) and the glycogen structure was normal. Glycogen content of the liver was normal histologically, as well as chemically; muscle was not available for biochemical glycogen determination. Enzyme studies (Dr. B. Brown, St. Louis; Dr. D. Wenger, Denver; Dr. R. Howell, Houston) on frozen liver and heart, cultured lung and diaphragm fibroblasts yielded normal levels of branching enzyme, debranching enzyme, glucose-6-phosphatase, phosphorylase, and alpha-glucosidase at pH 4 and pH 6.6, using maltose and glycogen as substrates.

Cardiomuscular and muscular glycogenosis resembling glycogenosis II (Pompe's Disease) with normal acid maltase (alpha-glucosidase) has been described on several occasions [1, 2, 3]. De Barsy et al. [2] described an 8-year-old boy with muscle weakness and a normal heart. In that case, acid maltase was normal in the muscle, but deficient in leukocytes. Two cousins described by Danon et al. [1, 3] were similar to our patient, yet in contrast to our case, they presented in their teens with proximal muscle weakness and a hypertrophic cardiomyopathy.

While cardiomyopathy is typical for glycogenosis II in infancy, the heart is usually not involved in childhood and adolescent variants of glycogenosis II. Our case is the first observation of cardiomuscular glycogenosis without a demonstrable enzyme deficiency is an infant. It is notable that all hitherto reported patients with this condition were boys, which raises the possibility of X-linked recessive inheritance, although classic glycogenosis II with acid maltase deficiency is transmitted as an autosomal recessive condition. However, this case may have other implications, as well. It may represent a hitherto unreported cause of congenital hydrops; thus, cardiomuscular glycogenosis might be included in the differential diagnosis of nonautoimmune hydrops congenitus.

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