

Thiamine (vitamin B1) supplementation does not reduce fasting blood homocysteine concentration in most homozygotes for homocystinuria

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Abstract

Homozygotes for homocystinuria due to cystathionine synthase (CS) deficiency accumulate homocysteine and methionine in their blood and tissues. High-dose pyridoxin, folic acid, vitamin B12, or betaine are therapeutical options to lower the elevated homocysteine concentration. These compounds stimulate the transsulfuration or remethylation of homocysteine. Despite such treatment, elevated blood homocysteine concentrations may persist in many homocystinurics. Therefore, it is warranted to study alternative regimen to reduce the blood homocysteine concentration in homocystinurics. Apart from entering the transsulfuration pathway, methionine can be catabolized via the transamination pathway, by conversion into 4-methylthio-2-oxobutyrate (MTOB), followed by oxidative decarboxylation of MTOB to 3-methylthiopropionate. Thiamine pyrophosphate, the active form of thiamine, is a cofactor of the supposed rate-limiting oxidative decarboxylation in the transamination of methionine. The effect of thiamine administered in 2 or 3 daily doses of 25 mg orally, was studied in nine homozygote CS deficient patients. Methionine levels decreased in 6 out of 9 patients. In 8 out of 9 patients, however, the levels of plasma homocysteine remained virtually unchanged, as did the serum transamination metabolites in all patients. We conclude that vitamin B1 cannot be used as an additional homocysteine-lowering treatment in most homozygotes for homocystinuria.

Keywords: Homocysteine; Homocystinuria; Thiamine; Transamination; Vitamin B1

1. Introduction

Patients with classic homocystinuria due to cystathionine synthase (CS) deficiency accumulate homocysteine and methionine in their blood and tissues. The disease is clinically characterized by arteriosclerosis, thromboembolism, eye-lens luxation, marfanoid features, osteoporosis, and mental retardation. Therapy is based on reduction of the hyperhomocysteinemia by stimulation of residual CS activity by high levels of the cofactor pyridoxal-5'-phosphate [1]. About 60% of these patients respond to pyridoxine. In poor or non-pyridoxine responsive patients, treatment can be extended with folic acid, vitamin B12 or betaine supplementation, which enhances the homocysteine remethylation (Fig. 1) [1]. As an ultimate op-

tion, dietary methionine restriction may be mandatory to reduce the blood homocysteine concentration. Despite this variety of therapeutical options, severely elevated homocysteine concentrations may persist in homozygotes for homocystinuria [2,3]. It is therefore warranted to study additional possibilities to reduce the blood homocysteine concentration in homocystinurics.

Apart from entering the transsulfuration pathway, methionine can also be degraded via the transamination pathway by conversion into 4-methylthio-2-oxobutyrate (MTOB), followed by oxidative decarboxylation of MTOB to 3-methylthiopropionate (Fig. 1) [4]. In the latter reaction thiamine pyrophosphate, the active form of thiamine, is a cofactor.

We previously demonstrated in an *in vitro* study that thiamine pyrophosphate stimulates the degradation of methionine via the transamination pathway in rat liver ho-

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mogenates [5]. The same result was obtained in human liver homogenates (Blom; unpublished results). In this study, the methionine and homocysteine-lowering effect of thiamine (vitamin B1) was tested in nine homozygote CS deficient patients.

2. Materials and methods

2.1. Homozygotes for homocystinuria due to CS deficiency

The criteria for selection of homozygotes for homocystinuria to enter this study were: regularly visiting our hospital, known with good compliance to their homocysteine-lowering treatment, prolonged elevated fasting homocysteine levels in blood despite homocysteine-lowering treatment for at least 2 yr, and at least 14 yr of age. Homozygosity for homocystinuria in nine patients was proven by hypermethioninemia, severe hyperhomocysteinemia as well as a near to absent CS activity in cultured skin fibroblasts [3]. None of them were on a regimen of methionine restriction, except one (patient number 9; Table 1). In addition to their conventional homocysteine-lowering treatment, six patients received 25 mg of thiamine hydrochloride for 6 weeks three times daily and three patients twice daily (Table 1: patient number 2, 6 and 7). The latter three patients received only in total 50 mg thiamine hydrochloride conforming with their conventional homo-

cysteine-lowering treatment which was also twice daily. Patients 3 and 4 are brothers, as well as patients 6 and 7 (Table 1).

2.2. Methionine and homocysteine assay techniques

Fasting EDTA venous blood samples were centrifuged immediately after puncture and stored at -20°C until analysis. Methionine concentrations were determined on a LC 2000 amino acid analyser (Biotronik Wissenschaftliche Geräte, Munich, Germany) [6]. The total homocysteine concentrations (free plus protein-bound) were measured by a technique based on high-performance liquid chromatography (HPLC) and fluorescent detection [7].

2.3. Transamination metabolites assay techniques

The degradation of methionine via the transamination pathway before and after treatment was studied by quantification in serum by the sum of the transamination metabolites 4-methylthio-2-oxobutyrate and the mixed disulfides of methanethiol (R-S-S-CH_3). The blood concentrations of these metabolites are low and near the detection limit, and because of reasons of accuracy, we prefer to measure the total sum of these transamination metabolites. This technique is based on gas chromatography (Packard type 429; Packard-Becker, Delft, the Netherlands), supplied with a sulfur-specific flame-photometric

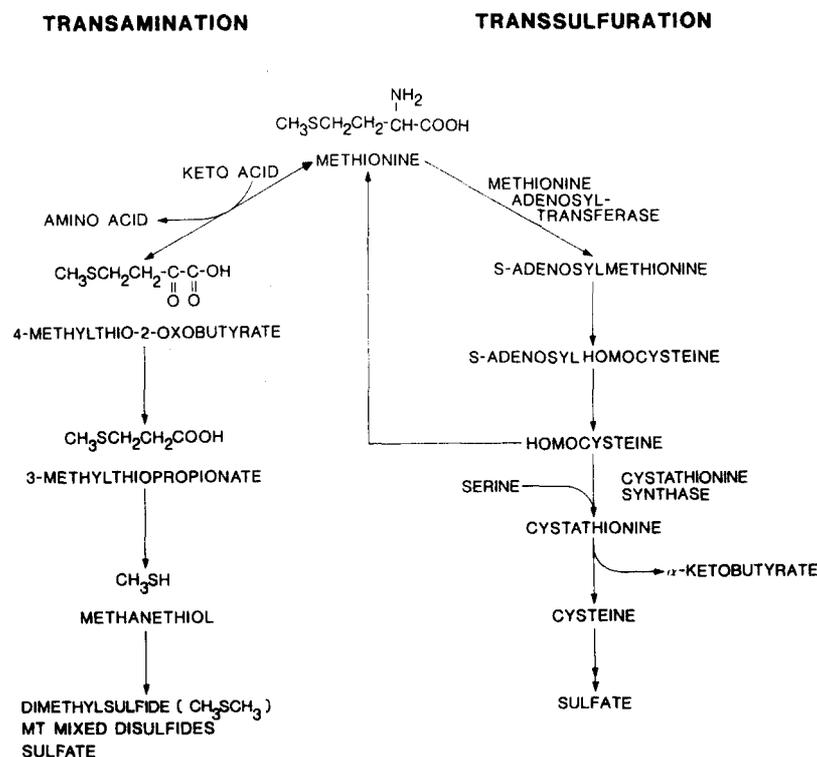


Fig. 1. Metabolism of methionine.

Table 1

The fasting blood concentrations of total homocysteine, methionine, thiamine (vitamin B1) and transamination metabolites in nine cystathionine synthase deficient patients before (-B1) and after (+B1) six weeks of thiamine treatment

Patient	Homocysteine ($\mu\text{mol/l}$)		Methionine ($\mu\text{mol/l}$)		Thiamine nmol/l		Transamination ($\mu\text{mol/l}$)		Therapy
	- B1	+ B1	- B1	+ B1	- B1	+ B1	- B1	+ B1	
1.	143	48	98	30	98	170	0.3	0.3	750B6, B12
2.	46	49	43	42	96	130	0.3	0.3	400B6, FA, B12
3.	81	82	291	123	110	190	0.6	0.3	750B6, FA, betaine
4.	82	79	94	54	120	210	bd	bd	750B6, FA, betaine
5.	58	56	161	58	100	210	0.3	0.3	750B6, FA, betaine
6.	113	101	222	93	np	np	0.4	0.2	500B6, FA, betaine
7.	82	120	139	188	np	210	bd	bd	500B6, FA, betaine
8.	85	103	57	72	110	np	0.3	0.3	750B6, FA, B12, betaine
9.	128	109	643	490	230	330	1.0	0.7	250B6, FA, betaine, MPR
mean \pm SD	91 \pm 25	83 \pm 22	194 \pm 127	128 \pm 94	123 \pm 30	207 \pm 38			
normal	5 to 18		16 to 47		47 to 142		bd to 1.2		

Vitamin B6 (B6) was given in a daily dose in mg as indicated; folic acid (FA) was given in a daily dose of 5 mg; vitamin B12 (B12) was given in a two monthly dose of 1 mg intramuscularly; betaine was given in a daily dose of 6 g; methionine poor regimen (MPR); np, not performed; bd, below level of detection.

detector (Packard, model 906) [8,9]. Details of the transamination metabolites measurement have been reported previously [10].

2.4. Vitamin B1 measurements

Total thiamine concentrations were determined by a modified procedure of the reversed-phase ion-pair HPLC technique described by Wienders and Mink [11]. Whole blood specimens are deproteinized with perchloric acid, followed by acid phosphatase hydrolysis of thiamine tri-, di- and monophosphate to thiamine, and post-column derivatisation of total thiamine with $\text{K}_3\text{Fe}(\text{CN})_6$ to thiochrome which is quantified fluorimetrically at excitation and emission wave lengths of 364 nm and 462 nm, respectively. Due to preparation steps prior to HPLC, total analysis time of a blood specimen takes approx. 20 h while the HPLC run itself, requires less than 10 min. The lower limit of detection of the technique is 2.3 nmol thiamine/l and excellent linear standard dose-responses is obtained up to 400 nmol/l. At mean concentrations of 123 nmol/l, the precision of the technique revealed intra-assay and inter-assay coefficients of variation of 4.8% (9 assay runs) and 6.8% (100 consecutive assay runs), respectively.

2.5. Statistical analysis

Results are given as mean \pm SD. The two tailed Wilcoxon rank sum test was used in assessing statistical significance.

3. Results

The mean age \pm SD of all 9 homozygotes for homocystinuria was 24.4 \pm 6.2 yr (range 14 to 34 yr); the mean \pm SD duration of homocysteine-lowering treatment

was 9.0 \pm 2.4 yr (range 5 to 17 yr). The mean \pm SD body weight was 72.3 \pm 10.6 kg (range 52.5 to 88.0 kg) and the mean \pm SD mg thiamine dosage per kg body wt. for the patients was 0.90 \pm 0.25 mg thiamine supplementation per kg body weight (range 0.48 to 1.43).

The vitamin B1 plasma concentration in the homozygotes for homocystinuria increased from 123 \pm 30 (mean \pm SD) nmol/l ($n = 7$) before thiamine treatment to 207 \pm 38 nmol/l ($n = 7$) after six weeks of thiamine treatment (Table 1). The fasting blood methionine concentration decreased from 194 \pm 127 $\mu\text{mol/l}$ (mean \pm SD) before thiamine treatment to 128 \pm 94 $\mu\text{mol/l}$ after thiamine treatment ($n = 9$) ($P < 0.06$). The mean fasting plasma homocysteine concentrations did not differ significantly before and after thiamine therapy, i.e. 91 \pm 25 $\mu\text{mol/l}$ vs. 83 \pm 22 $\mu\text{mol/l}$. Only one out of the nine thiamine treated patients showed a considerable lowering of the homocysteine concentration simultaneously with a major decrease of the methionine level (Table 1, patient number 1). Currently, this patient continues the vitamin B1 supplementation for 3 yr, and the basal total homocysteine concentration at the most recent determination is now as low as 34 $\mu\text{mol/l}$. The level of the transamination metabolites after thiamine did not increase in the patients. In fact, these levels remained low or undetectable in six patients and even decreased slightly in three other patients (Table 1). Even in the single patient with a decrease of the homocysteine concentration since the start with thiamine supplementation, the level of the transamination metabolites remained unchanged.

4. Discussion

Methionine degradation via the transamination pathway occurs in human but is probably of minor quantitative importance [10]. However, patients with hypermethionine-

mia due to methionine adenosyltransferase (MAT) deficiency do degrade quantitative amounts of methionine via the transamination pathway [12,16], whereas patients with hypermethioninemia due to cystathionine synthase (CS) deficiency do not, despite elevated methionine levels [6]. The functional impairment of methionine transamination may be due to the different biochemical level of the blockade in the methionine metabolism or due to the treatment of CS deficient patients by pharmacological amounts of vitamins, in particular pyridoxine [6]. The present study was performed because of the theoretical possibility to lower homocysteine concentrations by enhancement of methionine degradation via the transamination pathway in CS deficient patients.

Previous *in vitro* studies with rats [5] and human homogenates [Blom H, unpublished data] have shown that thiamine pyrophosphate stimulated the methionine degradation via the transamination pathway 2.5 fold. This active form of thiamine is a cofactor of the branched-chain 2-oxo-acid dehydrogenase complex which catalyses the oxydative decarboxylation of 4-methylthio-2-oxobutyrate into 3-methylthiopropionate [13,14]. This decarboxylation is supposed to be the rate-limiting reaction in the transamination of methionine [4].

Thiamine was administered in 9 homozygotes for homocystinuria in addition to their conventional homocysteine-lowering treatment. All these patients except one, demonstrated virtually unchanged levels of plasma homocysteine and serum transamination metabolites, despite the observed reduction of their methionine concentrations. Very recently, it has been reported that transamination in hypermethioninemic children is abnormally elevated only when plasma methionine levels exceeded 300 or 350 μM [15,16]. In the present study, only patient number 9 (Table 1) has a methionine level above this methionine level, and indeed, his transamination metabolites were higher than in the other 8 investigated homozygotes for homocystinuria, but still much lower than those of MAT deficient patients with comparable methionine levels [6,16]. But even his serum transamination metabolites concentration did not increase after the supplementation of thiamine.

We have no straightforward explanation for the reduction of the methionine concentration in most of the homocystinuric patients due to thiamine supplementation. The flux of methionine degradation through the transamination pathway can be decreased by glutamine, glutamic acid, alanine or leucine in the presence of 4-methylthio-2-oxobutyrate [17]. Thiamine is involved as a cofactor in the oxydative decarboxylation in the degradation of many amino acids, including the four mentioned above. Theoretically, thiamine administration may decrease the levels of glutamine, glutamic acid, alanine and leucine, and indirectly stimulate methionine degradation via its transamination pathway. However, the serum transamination metabo-

lites of methionine were not elevated during thiamine administration. But, the levels of these metabolites may have remained unchanged despite an increased methionine degradation through the transamination pathway. Studies using stable isotopes of methionine could clarify this matter.

In conclusion, orally administered thiamine lowered the homocysteine level in only one out of the nine homocystinuric patients studied despite the reduction of the methionine concentration in most patients. Therefore, vitamin B1 appears to be only a minor alternative option for homocysteine-lowering treatment next to vitamin B6, B12, folic acid, and betaine in homozygotes for homocystinuria.

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