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Vitamin supplementation reduces the level of homocysteine in the urine of autistic children

Joanna Kałużna-Czaplińska*, Monika Michalska, Jacek Rynkowski

Institute of General and Ecological Chemistry, Technical University of Lodz, 90-924 Lodz, Poland

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Abstract

Significant differences in homocysteine levels in the urine of autistic children are observed. We hypothesized that vitamin supplementation might reduce the level of urinary homocysteine. To rationalize such a hypothesis, analyses were performed using the gas chromatography/mass spectrometry method. The homocysteine level in the urine of autistic children was measured twice: (1) before vitamin supplementation (group C, 30 autistic children) and (2) after supplementation, with either folic acid and vitamins B_6 and B_{12} (group A1, 24 autistic children) or vitamins B_6 and B_{12} alone (group A2, 6 autistic children). The homocysteine level in the urine of autistic children before vitamin supplementation was 2.41 ± 1.10 mmol/mol creatinine (mean ± SD difference). After treatment, the homocysteine level was reduced to 1.13 ± 0.44 and 1.33 ± 0.39 mmol/mol creatinine for A1 and A2 groups, respectively. The intake of vitamins B_6 and B_{12}, together with folic acid, was found to be more effective in lowering the levels of urinary homocysteine than the intake of vitamins B_6 and B_{12} alone. Our findings may lead to the recommendation of including vitamins B_6 and B_{12} together with folic acid supplementation in the diets of children with autism.

Keywords: Autism; Children; Homocysteine; Supplementation; Urine; Vitamin B_6; Vitamin B_{12}; Folic acid
Abbreviations: GC/MS, gas chromatography/mass spectrometry.

1. Introduction

Autism is a complex metabolic disorder involving multiple organ systems, primarily the neurologic, immunologic, and gastrointestinal systems. Children with autism most commonly come under clinical attention between the second and third year of life. There are numerous theories as to the specific causes of autism, but they are not proved as of yet [1-3]. Many studies have reported that autism has a broad, multifactorial etiology, where the predisposing factors include toxic chemicals, antioxidant insufficiencies, genetic susceptibility, and nutrients [4]. Nutrition plays an important role in the maintenance of physical growth and development during a child's life. Many children with developmental disorders show abnormalities in the levels of amino acids in the body [5,6]. In recent years, metabolic biomarkers such as amino acids have been identified in children with different disorders [7,8].

Amino acids, including homocysteine, are eliminated in urine and provide essential data about diets and the functioning of the alimentary system. An improper diet and a poor functioning of the digestive system can strongly influence the intensity of autistic symptoms [9,10]. In humans, homocysteine is an amino acid derived from methionine. Homocysteine is metabolized via 2 pathways: either remethylation to methionine or transsulfuration to cysteine [11]. A defect in either of these pathways leads to an accumulation of homocysteine. Remethylation is a process that involves folic acid and vitamin B_{12},

* Corresponding author. Tel.: +48 426313091; fax: +48 426313128.
E-mail address: jkaluzna@p.lodz.pl (J. Kałużna-Czaplińska).

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whereas transsulfuration involves vitamin B_6. Vitamins B_6 and B_{12}, as well as folic acid, are necessary for lowering the homocysteine levels. Improper dietary intakes of these nutrients can lead to vitamin deficiencies, which can result in elevated levels of homocysteine. Folic acid must be included in the diet because humans cannot produce it [12].

Pasca et al [13] were the first to find relationships between the levels of homocysteine in serum of autistic children and Vitamin B_{12} deficiency. So far, the relationships between the levels of urinary homocysteine in autistic vs nonautistic children, as well as the effects of vitamin supplementation on the levels of homocysteine, have not been studied in detail.

We hypothesized that vitamin supplementation can reduce the levels of homocysteine in the urine of autistic children. To rationalize this, we reported quantitative data concerning the levels of homocysteine in autistic and nonautistic children. Moreover, we compared the levels of homocysteine in autistic children before and after treatment with vitamins B_6, B_{12}, and folic acid.

2. Methods and materials

2.1. Study population and sample collection

This study was restricted to the children diagnosed with autism in compliance with the criteria detailed in the *Diagnostic and Statistical Manual of Mental Disorders* [14]. All autistic children were assessed and diagnosed by clinicians specializing in the diagnosis and management of autistic children from the Navicula Centre in Lodz, Poland. In the case of all autistic subjects, psychomotor hyperactivity, repeated behaviors, mood disorders, aggression attacks, and disorders of social relationships were diagnosed. The first morning, urine samples were collected from 30 autistic children (4-11 years) and from 21 nonautistic children (4-11 years).

This study was approved by the local research ethics committee and conducted according to the Declaration of Helsinki and with the family's written consent.

2.2. Dietary intakes

For each autistic child, the homocysteine level in urine was measured twice: (1) before vitamin supplementation, when none of the children received folic acid and vitamins B_6 and B_{12} in their diet; and (2) 3 months after receiving either folic acid and vitamins B_6 and B_{12} (24 autistic children) or vitamins B_6 and B_{12} alone (6 autistic children). Children were supplemented daily with vitamins B_6, B_{12}, and folic acid in the dose of 200 mg, 1.2 μg, and 400 μg, respectively. All children followed a sugar-free diet. For comparison, a group of 21 nonautistic children (4-11 years) was included.

2.3. Gas chromatography/mass spectrometry analysis

Gas chromatography/mass spectrometry (GC/MS) urinary homocysteine analysis was performed using the method described previously [15] with some modification.

The homocysteine in urine was determined by GC/MS firm Agilent Technology (Santa Clara, Calif, USA) 6890 N Network GC System, 5973 Network Mass Selective. Capillary column HP-5MS (Agilent Technology; 30 m × 0.25 mm ID; film thickness, 0.25 μm) was installed in the gas chromatograph and inserted directly into the ion source of the mass spectrometer. The oven temperature of 100°C (1 minute) was then programed to 180°C at 20°C per minute then programed to 220°C at 10°C per minute. The injector temperatures were 250°C, and the transfer line was 280°C. Helium was used as a carrier gas at a flow rate of 0.9 mL/min. To confirm the mass fragments of the derivatives, data were obtained in full scan mode in the scan range from m/z (mass-to-charge ratio) 50 to 500.

Homocysteine needed to be extracted from the urine samples and derivatized before the chromatographic analysis. In this method, homocysteine was derivatized and extracted simultaneously. Added to 0.5 mL of the urine sample was 2-μg/mL^{-1} octadecane. Then, it was derivatized with 0.3-mL ethanol, 0.1-mL pyridine, 60-μL ethylchloroformate, and 0.6-mL chloroform. All vials were placed on an orbital shaker at room temperature (23°C ± 1°C) for 10 minutes. The organic layer of 2 μL was injected to the GC/MS.

Concentrations of creatinine were determined chromatographically according to the procedure described in detail elsewhere [16]. The creatinine in urine was determined by a high-performance liquid chromatography firm Agilent Technology 1100 series LC chromatographic system equipped with a vacuum degasser (G1379A), quaternary pump (G1310A), and ultraviolet/visible detector (G1314A). The analyses were performed on a column (4.6 × 150 mm, 5-μm Elipse XDB C-18; Agilent Technologies). The mobile phase was 98:2 (vol/vol), 15-mmol/L, pH 7.4 phosphate buffer acetonitrile. The flow rate was 1 mL/min; the temperature, 25°C; and the analytic wavelength, 234 nm. A 20-μL sample of urine, diluted 1:500, was injected onto the high-performance liquid chromatography.

2.4. Statistical analyses

Data were statistically evaluated using a statistical analysis package (StatSoft, Polska STATISTICA, version 9.0; Tulsa, Okla, USA). The Shapiro-Wilk test was used to check for normal distribution of the data. Data are expressed as means ± SD difference. The Scheffe test was used to determine differences in single variables between mean values for homocysteine in the group of nonautistic children and the 3 experimental groups. The level of statistical significance was defined as $P < .05$.