Neuroendocrinology Letters ISSN 0172–780X

ORIGINAL ARTICLE

Treatment of autism spectrum children with thiamine tetrahydrofurfuryl disulfide: A pilot study

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Submitted: *Febrary 17, 2002*
Accepted: *May 24, 2002*

Key words: **Autism; thiamine tetrahydrofurfuryl disulfide; urinary arsenic; sulfur metabolism**

*Neuroendocrinology Letters 2002; **23**:303–308 pii: NEL230402A02 Copyright © Neuroendocrinology Letters 2002*

Abstract

OBJECTIVES: In a Pilot Study, the clinical and biochemical effects of thiamine tetrahydrofurfuryl disulfide (TTFD) on autistic spectrum children were investigated. **SUBJECTS AND METHODS**: Ten children were studied. Diagnosis was confirmed through the use of form E2, a computer assessed symptom score. For practical reasons, TTFD was administered twice daily for two months in the form of rectal suppositories, each containing 50 mg of TTFD. Symptomatic responses were determined through the use of the computer assessed Autism Treatment Evaluation Checklist (ATEC) forms*. The erythrocyte transketolase (TKA) and thiamine pyrophosphate effect (TPPE), were measured at outset and on completion of the study to document intracellular thiamine deficiency. Urines from patients were examined at outset, after 30 days and after 60 days of treatment and the concentrations of SH-reactive metals, total protein, sulfate, sulfite, thiosulfate and thiocyanate were determined. The concentrations of metals in hair were also determined. **RESULTS**: At the beginning of the study thiamine deficiency was observed in 3 out of the 10 patients. Out of 10 patients, 6 had initial urine samples containing arsenic in greater concentration than healthy controls. Traces of mercury were seen in urines from all of these autistic children. Following administration of TTFD an increase in cadmium was seen in 2 children and in lead in one child. Nickel was increased in the urine of one patient during treatment. Sulfur metabolites in urine did not differ from those measured in healthy children. **CONCLUSIONS**: Thiamine tetrahydrofurfuryl disulfide appears to have a beneficial clinical effect on some autistic children, since 8 of the 10 children improved clinically. We obtained evidence of an association of this increasingly occurring disease with presence of urinary SH-reactive metals, arsenic in particular.

** Both the E2 and ATEC forms were designed at, and supplied by, the Autism Research Institute, 4182, Adams Avenue, San Diego CA 92116.*

ABBREVIATIONS:

TTFD	Thiamine tetrahydrofurfuryl disulfide
TKA	Erythrocyte transketolase
TPPE	Thiamine pyrophosphate effect on TKA
ATEC	Autism Treatment Evaluation Checklist
Delta ATEC	Final ATEC score subtracted from baseline ATEC

Introduction

The hypothesis that TTFD might have a beneficial effect on autistic spectrum disease was suggested from studies by Waring and associates who reported sulfur depletion in the plasma [1] of these children and abnormal concentrations of sulfur in their urine [2]. One of the urinary abnormalities was a decrease in the concentration of thiocyanate and these authors hypothesized that this might be due to decreased activity in the enzyme rhodanese that converts toxic cyanide ions to non-toxic thiocyanate. Japanese investigators [3] had previously shown that thiamine disulfides partially protected pretreated mice from cyanide poisoning by increasing the activity of rhodanese. In addition, it was hypothesized that TTFD would act as a donor of sulfate since sulfur depletion had been observed in autistic children [1].

Although TTFD is usually given by mouth in capsules, autistic children are notorious for the difficulties involved with oral administration. When we attempted to administer the powdered contents of a capsule to any children of this age, the excessively unpalatable taste caused instant rejection. It was then decided to provide the TTFD in the form of rectal suppositories.

Since there is no reliable biologic test to indicate treatment effect in autistic patients, the form E2 was used to confirm the diagnosis and the ATEC forms used to measure the response to treatment.

Patients and methods

Eight boys and two girls, with ages ranging from 3 to 8 years, were treated with rectally administered TTFD for 60 days in a pilot study. Patients were included in the study based on diagnosis made by a clinical specialist in the field. This diagnosis was confirmed later through the E2 diagnostic form [4]. The study protocol was reviewed by the Institutional Review Board of the American College for Advancement in Medicine.

The parents were asked to sign an informed consent after they had been given detailed information by verbal and written communication. They were then asked to fill in form E2. The completed form for each patient was mailed to the Autism Research Institute for processing. Each child had a suppository, containing 50 mg of TTFD, inserted twice a day in addition to any current treatment that was allowed to continue. Clinical evaluation was achieved by the use of the computer assessed Autism Treatment Checklist forms [5] that were filled in by the parents at baseline and at the end of the study.

Biochemical studies

1. Urinary analyses

Urinary samples from all the patients were examined at baseline, after 30 days and after 60 days of TTFD treatment. In addition, urinary samples from 19 age-matched healthy children were collected as controls. Each of the specimens was analyzed for total arsenic, lead, mercury, cadmium, nickel, total protein, sulfate, sulfite, thiosulfate and thiocyanate.

Urinary arsenic, lead and cadmium were measured using a Leeman DRE (Direct Reading Echelle) inductively coiled plasma emission spectrometer [6]. Mercury was analyzed using a Leeman PS 200 II automated analyzer using the principles of Hatch and Ott [7]. For analysis of sulfate, sulfite, thiosulfate, thiocyanate and total protein, the urinary samples were collected in the presence of 10% thymol in isopropanol (0.5 ml/100ml urine) and stored at – 20° C until the time of analysis. Each sample was coded by third party for blind analysis.

For the determination of sulfur metabolites, urinary samples were thawed at room temperature and sediments removed by centrifugation. The specific gravity and pH of each sample was adjusted to 1.022 and 6.4 respectively. To remove interfering substances, freshly distilled diethyl ether was saturated with deionized water at room temperature in a separating funnel for one hour. After removing the excess water, the ether was mixed with pure chloroform (95:5) and added to the urine samples for five minutes. The ether layer was removed and the samples incubated at 37°C for one hour before subjecting them for analyses.

Concentrations of inorganic sulfate were determined by the turbidimetric analysis of Lundquist *et al.*[8]. The calibration curve in triplicate was constructed each time the assay was performed. In a preliminary experiment, using ^{35}S-Na_2SO_4 spiked samples, recovery of sulfate was found to be 93 +/–4% (N=6). Urinary sulfite was determined using high-pressure liquid chromatography by a modification of the method of Togawa *et al.*[9]. Thiosulfate was measured by gas chromatography and mass spectroscopy by the method of Kage *et al.* [10] and thiocyanate by the method of Vesey *et al.* [11].

Urinary total protein was assayed by the method of Wantanabe *et al.* [12] after removal of interfering substances by precipitating the protein with sodium deoxycholate and trichloracetic acid. Using ^{125}I-labeled human serum albumin, recovery of protein was found to be 87+/–5% (n = 6).

2. Hair analysis.

Hair samples were collected at the beginning and at the end of the study. One gram of hair was cut from the nape of the neck, then dissolved in trace metal-free nitric acid and diluted with 10 ml deionized water. Analysis was performed with a Leeman PS 2000 inductively coiled emission plasma spectrometer and compared to standards traceable to the National Bureau of Standards [6].

3. Erythrocyte transketolase

Erythrocyte transketolase studies [13] were performed at the beginning and again after 60 days of TTFD treatment. This *in vitro* test is the only laboratory test that reliably measures activity of thiamine pyrophosphate in cells. It is reported as the baseline activity of the enzyme (TKA) before the addition of thiamine pyrophosphate (TPP). The TKA is obtained by measuring the amount of product formed per unit of time. After the addition of TPP, the reaction is repeated. Any acceleration in product synthesis indicates the degree of prior cofactor desaturation and is reported as an increased percentage over baseline activity of the transketolase, the TPP effect (TPPE).

Results

This small group of autistic children was heterogeneous in their symptomatology as shown by their E2 scores that ranged from –31 to +25 (Table I). Following the administration of TTFD, the scores of all except 2 patients improved. One patient (MiS) was a 6-year-old girl who had been treated for a seizure disorder and autistic symptoms prior to being accepted for the study. Her E2 score was –31 and she showed no clinical improvement (Delta ATEC score –4). Another child (MaS) was a 3-year old boy with an E2 score of –14. His Delta ATEC score (–19) indicated increased symptomatology.

ATEC, it became clear that the most severely affected patients had the best symptomatic responses (*Figure 1*). Interestingly, in 9 of our patients, the parents reported an odor from their child described as resembling that from a skunk. This was invariably an odor from stools, but in one or two individuals the odor was noted from urine or sweat.

Table II shows the concentrations of arsenic in the urine and hair of all 10 patients. The small number of patients made statistical analysis impossible.

In addition to arsenic, patient MK showed lead in start and 30 day urinary samples (34.3 and 83.1 μg Pb/g creatinine respectively: (normal value < 20) and 6.4 μg Cd/g creatinine of cadmium in "0" urine (normal value < 4). Although traces of mercury were present in urinary samples of all 10 children, the "0" sample in patient JK revealed 9.0 μg Hg/g creatinine, which then decreased to 3.2 μg Hg/g creatinine in the 60 day urine. The 60 day urinary sample of patient MaS contained 104 μg Hg/g creatinine, following TTFD treatment. Increases in nickel were seen in only one patient. In addition to arsenic and increased concentrations of mercury, following TTFD treatment the urine of

Table I.

Patients	Speech	Behavior	E2 Total	Delta ATEC
MiS	–4	–27	–31	–4
MaS	0	–14	–14	–19
JV	0	–11	–11	7
AS	–1	–7	–8	17
AF	4	–11	–7	8
MK	0	–8	–8	14
JK	0	–8	–8	48
SMcC	3	–3	0	33
NW	6	7	13	38
TS	4	21	25	62

Diagnostic E2 and Delta ATEC score for each of the 10 autistic children. The Delta ATEC is calculated by subtracting the 60-day score from that obtained at the start of the study.

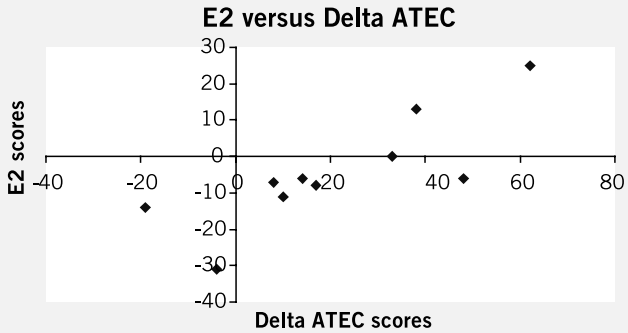

Figure 1. E2 scores in 10 autistic children plotted against their Delta ATEC scores determined after treatment. Delta ATEC is calculated by subtracting the ATEC score at 60 days from that obtained at the beginning of the study.

TABLE II Urinary and Hair Arsenic

	Urine(< 40 μg/g creatinine)			Hair (< 2.0parts per million)	
Patient	**Start**	**30 days**	**60 days**	**Start**	**60 days**
MiS	39	51.7	53	1.5	2.22
MaS	75.9	70.8	44.7	.09	2.19
JV	39.4	15.5	38.7	2.48	1.37
AS	288	58.3	48.1	1.55	3.5
AF	25	56.5	56.2	2.97	3.8
MK	125	107	65.1	1.66	1.08
JK	35.5	137	93.7	.76	2.62
SMcC	50.7	67	38	2.07	3.13
NW	41.1	47.8	17.7	.81	1.68
TS	44.9	38.6	65.4	2.28	1.81
MEAN	76.5+/–79.7	65.0+/–34.5	52.1+/–20.3	1.62+/–0.88	2.34+/– 0.91
Controls	32.5+/–17	N=19			

Urinary and hair arsenic measured in 10 autistic children at the start of the study and after 60 days of treatment.

Table III. Urinary sulfur metabolites

	CONTROLS	PATIENTS		
	Mean	**Baseline**	**30 Day**	**60 Day**
Protein	85.9+/–45.6	88.0+/–76.8	72.3+/–65.6	62.2+/–44.0
Total sulfate	7742.7+/–3789.5	6543.1+/–4524	7134.3+/–4631	5946.2+/–4278
Sulfite	50.9+/–84.6	28.1+/–57.9	47.2+/–77.6	16.9+/–24.7
Thiosulfate	39.6+/–26.1	25.8+/–19.3	25.8+/–15.7	25.6+/–24.5
Thiocyanate	11.1+/–14.4	24.4+/–14.4	24.9+/–21.1	29.3+/–28.7

Urinary protein and sulfur metabolites measured in 10 autistic patients and 10 healthy children as controls.

Table IV

	Transketolase (42–86 mU/l/min)		Thiamine pyrophosphate effect (0–17%)	
Patients	**"0"**	**60 days**	**"0"**	**60 days**
MiS	74	90	8	0
MaS	55	77	9	4
JV	77	80	**18**	8
AS	74	83	5	11
AF	86	81	5	**30**
MK	87	66	3	11
JK	56	89	**41**	9
SMcC	78	80	10	15
NW	66	Not tested	5	Not tested
TS	43	76	**21**	4

Erythrocyte transketolase and thiamine pyrophosphate effect obtained from 10 autistic children at the beginning of the study and after 60 days of treatment.

patient JK contained 35 μg Ni/g creatinine at 30 days and 68.4 μg Ni/g creatinine at 60 days (normal value < 10). It may be relevant that this child had evidence of thiamine deficiency that was corrected as shown by results of the transketolase study after 60 days of TTFD treatment (Table IV).

Results of transketolase studies showed thiamine deficiency at the start of the study in 3 of the 10 patients and one after 60 days of treatment (Table IV).

Discussion

The most important result of this pilot study as shown by the use of the E2 and ATEC forms was improvement of autistic spectrum symptoms in 8 of 10 children. E2 Scores of –15 or higher, and rarely running as high as +30 or +40, are usually regarded as "autism," the common form that is occurring today in epidemic numbers (Rimland B. Personal Communication). The higher the score on the E2, the closer the patient resembles classic autism as described by Kanner [14]. The E2 score of –31 in one patient (MiS) fell below this range, indicating that her symptoms were not within the autistic spectrum and in another (MaS) the score of –14 was just at the borderline of the spectrum as judged by E2. The ATEC form uses 4 scales of measurement. Studies at the Autistic Research Institute showed that its reliability, using the Pearson split-half (internal consistency) coefficient for uncorrected r for speech was .920, for sociability .836, for sensory/cognitive awareness .875, and for behavior .815. For the total ATEC score it was .942. In the absence of a reliable biologic test, the E2 and ATEC forms have been used widely by investigators throughout the world and have become acceptable in the clinical evaluation of autism. Previous diagnostic instruments were considered to be insufficiently sensitive to show change in an individual resulting from treatment [15].

Our results, although preliminary, suggest that TTFD might be valuable in the treatment of this devastating and increasingly common disease in children. One patient (MS) showed a worsening of his symptoms during the study. There were no untoward effects observed in the other 9 patients except an unpleasant odor. Since this study was completed, we have continued to use TTFD in a clinical setting and, only in one case, the patient's symptoms increased and the parents discontinued the suppositories. Another disquieting symptom has also been observed in two patients, though none of our current study patients were affected. A perianal rash appeared after about a week of suppository treatment in two patients and, in each case, was extremely irritating to the child. After discontinuation of TTFD treatment, the rash disappeared within several days. Inserting suppositories with the same excipients, but without TTFD, in one patient did not cause skin eruption, suggesting that it was TTFD-specific.

Despite the small number of patients studied, the results indicate that SH-reactive metals are present in increased concentration in these autistic children. Arsenic was the metal that appeared to be most common since the majority of the patients had substantial concentrations in urine (Table II). The small number of patients and the wide variation of urinary arsenic concentration made a statistical analysis impossible, but the results seem to indicate decrease in values after 30 and 60 days of TTFD treatment. In this respect, it is interesting that 4 children had increased arsenic in hair at the start of the study and 6 children at the end of the study, representing excretion of arsenic into the hair. There were no consistent findings of other heavy metals in the hair. The abnormal results of TPPE, indicating intracellular thiamine deficiency in three patients may be important, but remains to be further investigated. In one patient (AF) the TPPE increased after 60 days of treatment. Although TTFD has not been previously administered to our knowledge rectally, it passes through cell membranes easily [3] and this finding remains unclear.

Allithiamine occurs naturally as an active principle of garlic [3] and TTFD is its synthetic counterpart. Its administration to humans and animals results in blood levels of thiamine that are much greater and more sustained than with the water soluble thiamine salts [16]. The mechanism of its therapeutic effect may be multiple. It is known that it increases the concentration of thiamine in the cytosol and that phosphorylation to thiamine pyrophosphate (TPP) and thiamine triphosphate (TTP) occurs in heart [17]. If this affects energy metabolism in the central nervous system it may be important since the formation of TTP is particularly important in this tissue [18].

Several investigators [19–21] have shown that lead, given experimentally to animals, was removed by administration of thiamine and the results from urinary analyses in our children suggest that TTFD treatment was associated with removal of arsenic and perhaps other SH-reactive metals. The prosthetic fragment derived from the reaction of TTFD in cell membranes is metabolized by a series of enzymes [22–26]. This mercaptan is a major metabolite of TTFD and is probably the source of the "skunk-like" odor that emanated from 9 of our 10 study children. The odor from the secretion of skunks is due to a mixture of mercaptans [27]. Thus TTFD may be a source of sulfur in sulfur-depleted autistic children [1].

Until now arsenic has not, to our knowledge, been considered as an important agent associated with autism. Lead, arsenic, mercury and cadmium are SH-reactive metals and their biochemical actions have similar effects, though their tissue distributions are different [28]. Like mercury, arsenic impairs cellular respiration through inhibition of mitochondrial enzymes. It causes uncoupling of oxidative phosphorylation by inhibition of sulfhydryl-group-containing cellular enzymes and substitution of phosphate with arsenate in "high-energy" compounds. It has been shown to block steps in the Krebs cycle [29]. Its concentration in urine is stable as a function of its ingestion [30]. Low concentrations of arsenic are found in foodstuffs [31] and another source may be from chromated copper arsenate leaching into the water table from pressure treated wood [32]. Metallothionein null mice may be more sensitive than wild-type mice to the effects of arsenic administration [33] and this suggests the possibility of a genetically determined enzyme defect in autistic children, making them more susceptible to effects of toxicants.

The results from analysis of the sulfur metabolites showed extreme variation in their concentrations in both patients and controls and we were unable to confirm the reported data on these metabolites by Waring and associate[2]. Since these autistic diseases vary in severity, and we are dealing with a different population, our failure to agree with the results reported by these authors [2] is not necessarily surprising. There is a growing consensus among clinical investigators that this type of autism is caused by a variety of biochemical abnormalities that manifest as similar symptomatology.

Conclusions

Treatment with TTFD containing suppositories for 60 days improved 8 out of 10 children with symptoms attributed to autistic spectrum disease. Urinary concentrations of arsenic were increased at the outset and during the study in the majority of the patients compared with those observed in healthy, age-matched controls. Increases of urinary cadmium, nickel, and lead were also found in some patients. Traces of urinary mercury were found in all 10 patients and there was a marked increase in release of this metal in the urine of one child (MaS) after 60 days of treatment. Thus, TTFD may be a valuable addition to therapy of this increasingly common disease and deserves further study.

Acknowledgements

This Pilot Study was supported by a grant from the Autism Research Center, San Diego. TTFD was used under FDA Independent Investigator License 11019.

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