

Tetrahydrobiopterin in the Treatment of Children With Autistic Disorder

A Double-blind Placebo-Controlled Crossover Study

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Abstract: Twelve children, all boys, aged 4 to 7 years, with a diagnosis of autistic disorder and low concentrations of spinal 6R-*l*-erythro-5,6,7,8-tetrahydrobiopterin (tetrahydrobiopterin) were selected to participate in a double-blind, randomized, placebo-controlled, crossover study. The children received a daily dose of 3 mg tetrahydrobiopterin per kilogram during 6 months alternating with placebo. Treatment-induced effects were assessed with the Childhood Autism Rating Scale every third month. The results showed small nonsignificant changes in the total scores of Childhood Autism Rating Scale after 3- and 6-month treatment. Post hoc analysis looking at the 3 core symptoms of autism, that is, social interaction, communication, and stereotyped behaviors, revealed a significant improvement of the social interaction score after 6 months of active treatment. In addition, a high positive correlation was found between response of the social interaction score and IQ. The results indicate a possible effect of tetrahydrobiopterin treatment.

(*J Clin Psychopharmacol* 2005;25:485–489)

The treatment alternatives of autistic disorder (AD) have shown to be effective only relative to nonspecific disabling symptoms, but social interactions and impaired communication have so far appeared to be less responsive to pharmacological treatment. An early diagnosis of AD and an early start of treatment, which is mainly based on education, training, and behavior management, are important and believed to improve the prognosis. Despite this, many children remain significantly impaired, and new treatment paradigms are warranted.

Tetrahydrobiopterin is an essential cofactor in the hydroxylation of phenylalanine, tyrosine, and tryptophan and therefore plays an important role in the biosynthesis of catecholamines and serotonin. It is also an enhancer of the

synaptic release of various neurotransmitters including catecholamines, serotonin, acetylcholine, glutamate and γ -aminobutyric acid.^{1,2} It has been shown that the endogenous production of tetrahydrobiopterin is reduced in autistic children, as compared with nonautistic children.³

Although not clearly shown, oral tetrahydrobiopterin treatment may affect the concentrations of neurotransmitters in the central nervous system.⁴ Tetrahydrobiopterin treatment would enhance both dopaminergic and serotonergic tone in the brain and have behavioral effects as it increases the synaptic release of neurotransmitters and peripherally influences the serum levels of phenylalanine and tyrosine.⁵

Recently, a number of reports have emerged in which tetrahydrobiopterin has been tested as a possible treatment for children with autism.^{4,6–9} The overall impression from these studies is that more than half of the subjects showed a moderate or larger improvement of autistic symptoms as measured by the Rating Scale for Abnormal Behavior in Children. However, all but 1 of the studies were open, and the results must be interpreted with care.

On the basis of the above results, a hypothesis was put forward that young children with AD and low tetrahydrobiopterin concentrations in the cerebrospinal fluid (CSF) would respond positively to treatment with this compound. Thus, a double-blind placebo-controlled crossover study was conducted to evaluate the efficacy of the drug. In addition, it was expected that the response rate could be correlated with concentrations of tetrahydrobiopterin in the CSF.

MATERIALS AND METHODS

Twenty-eight children with suspected AD, 2 girls and 26 boys, aged 3 to 7 years, were considered for the study. All children were outpatients from 4 different departments of child and adolescent psychiatry in Sweden. Some were newly assessed and treatment-naïve at the time of the study. The others had had traditional nonpharmacological training and education. All the children were medication-free apart from the study drug and lived at home with their families throughout the study period. They all attended their usual special education programs in their local school districts.

On entry to the study, all children were assessed and rated by child psychiatrists according to the Childhood Autism Rating Scale (CARS).¹⁰ Testing of IQ levels was performed by child psychologists according to the Griffiths

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Received August 12, 2004; accepted after revision June 17, 2005.

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ISSN: 0271-0749/05/2505-0485

DOI: 10.1097/01.jcp.0000177667.35016.e9

TABLE 1. Demographics

	Child	Age (y)	No. DSM-IV Criteria (Max 12)*	IQ†	CSF Tetrahydrobiopterin (mmol/mL)	CARS	Comorbidity	Family History‡
Group A	1	5.8	11	42	30	36.5	Fragile X	LD
	2	4.4	9	84	9	32.5	Mild hearing impairment	MR
	3	5.0	7	71	28	32	Celiac	MR
	4	4.4	9	93	25	36		MR
	5	5.2	9	58	19	37.5		PPD
	6	4.6	8	50	28	38		AN
Group B	7	3.8	8	52	28	42		
	8	4.4	10	52	30	39.5		OCD
	9	5.7	9	32	29	45		Autism
	10	7.0	12	33	29	48		MR, LD
	11	5.5	10	44	30	39.5		
	12	7.8	8	75	28	34.5	Genes associated with MEN II§	

DSM-IV indicates *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*; MEN, multiple endocrine neoplasia; LD, language delays; MR, mental retardation; PDD, pervasive developmental disorder; OCD, obsessive compulsive disorder; AN, anorexia nervosa.

*First 12 criteria.

†Griffiths test.

‡Mother has MEN.

§First- or second-degree relatives with either LD, MR including Down syndrome, PDD, OCD, or AN.

Developmental Scale.¹¹ Parents were asked about possible adverse events during the prenatal, perinatal, and postnatal periods as well as their family history. Objective information was also collected from hospital files. After the initial psychiatric and psychological testing, a laboratory investigation, including CSF analysis, to evaluate the concentration of tetrahydrobiopterin was performed.

The following inclusion criteria were used: (a) a diagnosis of AD according to the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*, (b) aged 3 to 7 years, (c) no previous history of asthma or (d) epilepsy, (e) an age-adjusted IQ score of 30 or more on Griffiths Developmental Scale, (f) no previous pharmacological treatment against AD, and (g) CSF tetrahydrobiopterin less than 30 pmol/mL. The criteria were selected to include children with AD without severe mental retardation and with low concentrations of tetrahydrobiopterin in CSF. The limit of tetrahydrobiopterin was initially set to 12 pmol/mL based on an earlier study³ where the CSF concentrations were significantly reduced in the autism group as compared with the control group. However, it was soon disclosed that the concentrations were much higher than that, because of a better handling of the samples (R Moulder, personal communication, May 2004). Therefore, the limit was changed to 30 pmol/mL. This change of protocol was done after initiation of the study when 5 of the children were analyzed, but before randomization and start of treatment. Patients with asthma and epilepsy were not included because of potential adverse influences of drug treatment of these disorders.

Three of the 28 children did not meet the criteria for AD, and 7 children had CSF tetrahydrobiopterin greater than 30 pmol/mL. The remaining 18 children, 1 girl and 17 boys, fulfilled the inclusion criteria. Six of them were treated in a

different dose schedule and therefore not possible to be analyzed in this part of the study. Their data will be included in a forthcoming paper on positron emission tomography imaging (Y Watanabe, personal communication, May 2004). The study group comprised 12 children. All were boys and ranged in age from 3.8 to 7.8 years. One child was Persian, and the other 11 were white. For demographic information, see Table 1.

The ethics committee of the medical faculty of Uppsala University, the ethics committee of the Karolinska Institute, Stockholm, and the Swedish Medical Products Agency approved the study. Before any study-related assessment was performed, the parents of each child gave their written informed consent.

Procedure

A double-blind, randomized, placebo-controlled, cross-over design was used. Children were assessed every third month using CARS and for side effects. They were randomly placed into 2 groups (A and B) and received 6 months of placebo or tetrahydrobiopterin treatment, respectively, and thereafter, treatment was crossed over. There was no washout period between tetrahydrobiopterin and placebo treatments.

Individual doses of tetrahydrobiopterin at 3 mg/kg of body weight were prescribed in capsule form (in single-dose pack) to be taken twice daily. The weight of each child was measured at inclusion, and the dosage remained constant throughout the study period. The placebo was prepared in identical capsules (shape, size, color, and taste). The capsules were administered with soft drinks, fruit juice, or water. A new batch of capsules was provided every third month. The compliance to prescribed therapy was confirmed by the

parents at the end of each period when remaining capsules were recollected and counted. The local hospital pharmacy produced the capsules and performed the randomization of the patients. The tetrahydrobiopterin substance was kindly supplied by Suntory Ltd (Pharmaceutical Division, Tokyo, Japan).

Outcome Measures

The CARS was considered as the primary outcome measure of the study. The scale comprises 15 variables covering different autistic symptoms. Each variable is graded on a 7-point scale between 1 and 4—the higher the score, the more severe the symptom. Usually, preschool children with AD have a total score of more than 30 out of possible 60 points. In the preparatory work of the study, 4 specialists in child and adolescent psychiatry were tested to assess the interrater reliability of the CARS. Two children with AD not included in this study were videotaped and reviewed. The reproducibility between the raters was acceptable with total CARS scores varying between 45.0 and 47.0 of the first child and between 31.5 and 35.5 of the second one. The assessors remained blinded throughout the study.

As a post hoc analysis, the variables were subclustered into 3 categories, that is, social interaction, communication, and stereotyped behaviors according to the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (APA, 1994). The clustered scaled variables of social interaction were impairment in human relationships (1), impairment in imitation of sounds, words, and movements (2), inappropriate affect (3), and peculiarities of visual responsiveness (no. 7). The variables of communicative impairment were peculiarities in relating to nonhuman objects (5), peculiarities of auditory responsiveness (8), impaired verbal communication (11), and impaired nonverbal communication (12). The variables of stereotyped and restricted behaviors were bizarre use of movements and persistence of stereotypes (4), resistance to environmental change (6), and anxiety reaction (10). Ratings were gathered at inclusion and every 3 months within the study period. In the event rating took place before a month before treatment, a new rating was carried out and used as the baseline value.

Side Effects

Every 3 months, parents were asked to report suspected side effects from the treatment displayed by the child. They were also systematically asked about the presence of stomach pain, vomiting, headaches, fatigue/drowsiness, agitation, sleeping problems, and skin rashes. Adverse effects were recorded as “never,” “sometimes,” or “often.”

Statistical Analysis

CARS data were analyzed with the Wilcoxon signed rank test to compare the tetrahydrobiopterin treatment period to the placebo treatment. A significance level of 5% was used. The scorings for active treatment periods of the 2 groups, A and B, were added and compared with the ones of placebo treatment. Baseline values were scorings from inclusion and 6 months, respectively. A linear regression analysis was performed using the least squares method to

calculate how the response of social interaction was affected by age, IQ (Griffiths Developmental Scale), and CSF tetrahydrobiopterin concentration.

RESULTS

All 12 children completed the tetrahydrobiopterin treatment study. Six children repeated their CARS assessment before start of treatment since more than 1 month passed between inclusion and start of treatment. In child 4, the scoring was performed 10 months before start of treatment but was not repeated. Apart from that, there was no protocol violation.

Drug Efficiency

There were no significant differences in the total scores of CARS after 3 or 6 months of treatment when comparing tetrahydrobiopterin to placebo. After 3 months, the CARS score decreased with 1.8 ± 3.0 (mean \pm SD) points during tetrahydrobiopterin treatment and with 1.1 ± 4.6 points during placebo, as compared with baseline values. After 6 months, the decrease was 2.1 ± 2.1 and 2.1 ± 4.3 , respectively. There was no difference in baseline values between the 2 groups, group A, 35.4 ± 2.6 ; and group B, 37.4 ± 7.1 ($P = 0.56$). However, as a post hoc analysis, when looking at the core symptoms of AD, a significant difference concerning the social interaction score after 6 months of treatment with 1.6 ± 1.1 on tetrahydrobiopterin and with 0.3 ± 1.4 points on placebo ($P = 0.04$) was found. No significant difference was found after 3 months. The baseline value for group A was 9.8 ± 0.6 , and for group B, 10.8 ± 1.6 ($P = 0.11$). The 2 other subscales showed no significant changes within treatment groups. The scorings improved more during the first 6 months than the last 6, despite placebo or active treatment, but the changes were not significant.

A regression analysis was performed to analyze the response rate of social interaction in association with different clinical variables at inclusion to the study. The response rate of social interaction was not significantly related to age ($R^2 = 0.01$, $P = 0.75$), CSF tetrahydrobiopterin concentrations ($R^2 = 0.25$, $P = 0.10$), or total CARS score ($R^2 = 0.27$, $P = 0.08$), but to IQ ($R^2 = 0.37$, $P = 0.04$; Fig. 1). There was also a negative significant correlation with IQ and total CARS score ($R^2 = 0.61$, $P = 0.003$).

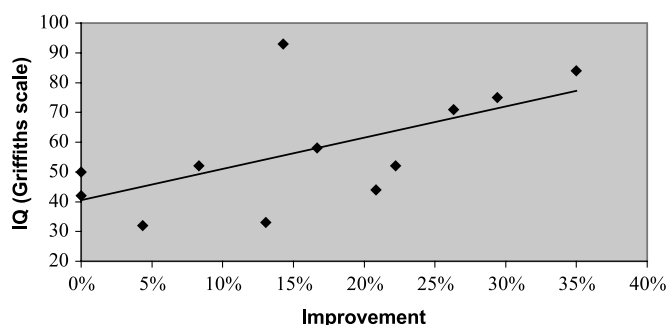


FIGURE 1. Correlation between IQ and improvement of social interaction after 6 months of tetrahydrobiopterin treatment ($n = 12$, $P = 0.04$).

Side Effects

Ten of the twelve children experienced possible side effects during the study. Most common were “agitation” (3 children during tetrahydrobiopterin and 3 during placebo) and “sleeping problems” (4 during tetrahydrobiopterin and 5 during placebo). No significant differences of reported side effects were found between periods of tetrahydrobiopterin or placebo treatment. The only serious event reported was a mild allergic reaction in 1 child which remitted spontaneously and was not considered to be tetrahydrobiopterin-related.

DISCUSSION

In this treatment study of young children with AD, tetrahydrobiopterin was not found to have an obvious positive effect. However, post hoc analysis showed a small but statistically significant improvement of the ability to interact socially, and a correlation was found between improvement of social interaction and IQ. The treatment was considered safe and well tolerated. All children completed the study.

There are important limitations of this study. One is the small sample size. It is also not clear how the concentrations of CSF tetrahydrobiopterin of the children included relate to healthy control subjects. The handling of the CSF samples was different compared with earlier studies, and hence, the qualification for inclusion was changed. Finally, the subscales of CARS are not validated. The strengths of the study are that it is randomized placebo-controlled and that the subjects were drug-naïve.

Earlier treatment studies with tetrahydrobiopterin mainly performed in Japan^{4,6-9,12} showed similar results to the present study. Four of these studies performed between 1985 and 1990 were open and could be influenced by placebo effect. The double-blind study of Naruse et al⁶ showed that 54% of the children in the tetrahydrobiopterin group and 31% in the placebo group responded to treatment. This difference was significant with the best response observed in children younger than 5 years. However, a correlation of age and response rate of social interaction found in the earlier tests was not found in the present study. This can be explained by the narrow age limits of the patients. A prominent period effect was also noted, where scorings improved more during the first period than the last irrespective of treatment.

The outcome measures in clinical drug trials of autism have recently been actively debated.^{13,14} Pharmacological treatment studies in AD are complicated by various factors including a multitude of symptom expression. Treatments developed are effective relative to certain disabling symptoms, but “core” problems appear less responsive to medications.¹⁵ Previous treatment studies with secretin have lifted the question of what to measure in autism drug trials.¹⁶ The lack of measures sensitive to critical clinical changes represents a serious deficiency in any approach to drug outcome evaluation in studies of AD. Most of the variables in CARS, with the exception of activity level, intellectual functioning, and general impression (variables 13, 14, and 15), correspond directly with the *Diagnostic and Statistical*

Manual of Mental Disorders, Fourth Edition variables. The categorization of 2 of these variables might be discussed. The first is imitation of sounds, words, and movements (variable 2), which could belong to both social interaction and communication. The second variable is anxiety reaction (variable 10), which can be considered to be a nonspecific symptom of AD. After consideration, it was decided to include these 2 variables in the analysis.

The rate of side effects in the present study was low, and no differences between placebo and active treatment were found.

CONCLUSIONS

In the validation of treatment effects, it seems prudent to differentiate drug effects on the core symptoms of AD. The effect of tetrahydrobiopterin treatment of AD was not pronounced but seemed to improve the impairments of social interaction in some high-functioning young children. This effect was seen without the presence of substantial side effects. More studies with different dosages are necessary to elucidate whether the effect of tetrahydrobiopterin can be improved.

ACKNOWLEDGMENTS

This research was supported by grants from the Subfemtomole Biorecognition Project, ICORP, Japan Science and Technology Agency (JST), The Swedish Research Council (grant 8645) the Sven Jerring Fund, Holmia insurance company, the Gillberg Foundation, the Samaritan Foundation, the Linnea and Josef Carlssons Foundation, and the child-neurology fund of Uppsala University. The authors thank Berit Lagerheim, MD, PhD, child psychiatrist; Hans Smedje, MD, PhD, child psychiatrist; and Johan Valtysson, MD, PhD, anesthesiologist, for their assistance in data collection; Hans Arinell for statistical analysis; Ann Svensson Allborg, Anna Karin Östling, Susanne Barr Carling, for Griffiths scaling; and Andrew Metcalfe for editorial support. We also thank Suntory, Ltd, for a generous gift of tetrahydrobiopterin.

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