Original article

Administration of secretin for autism alters dopamine metabolism in the central nervous system

Yoshihiro Toda\textsuperscript{a,\*}, Kenji Mori\textsuperscript{a}, Toshiaki Hashimoto\textsuperscript{b}, Masahito Miyazaki\textsuperscript{a}, Satoshi Nozaki\textsuperscript{c}, Yasuyoshi Watanabe\textsuperscript{c}, Yasuhiro Kuroda\textsuperscript{a}, Shoji Kagami\textsuperscript{a}

\textsuperscript{a}Department of Pediatrics, School of Medicine, University of Tokushima, 3-18-15, Karamoto-cho, Tokushima-shi, Tokushima 770-8503, Japan
\textsuperscript{b}Department of Education for the Disabled, Naruto University of Education, 748 Nakajima, Takashima, Naruto-cho, Naruto-shi, Tokushima 772-8502, Japan
\textsuperscript{c}Department of Physiology, Osaka City University Graduate School of Medicine, 1-4-3, Abenimachi, Abeno-ku, Osaka-shi, Osaka 545-8585, Japan

Received 20 October 2004; received in revised form 25 May 2005; accepted 25 May 2005

Abstract

We evaluated the clinical effects of intravenously administered secretin in 12 children with autism (age range: 4–6 years, median age: 9 years, boy:girl = 8:4). In addition, we investigated the association between improvement in symptoms and changes in the cerebrospinal fluid (CSF) homovanillic acid (HVA), 5-hydroxyindole-3-acetic acid (5-HIAA), and 6R-5,6,7,8-tetrahydro-L-biopterin (BH\textsubscript{4}) levels after administration. After administration of secretin, the Autism Diagnostic Interview-Revised (ADI-R) score improved in 7 of the 12 children. However, the score deteriorated in 2 of the 12 children (in the item of ‘restricted and repetitive, stereotyped interests and behaviors’). The HVA and BH\textsubscript{4} levels in CSF were increased in all children with improvement in the ADI-R score. In contrast, no patient without the elevation of the BH\textsubscript{4} level showed improvement in the score. These findings suggest that secretin activated metabolic turnover of dopamine in the central nervous system via BH\textsubscript{4}, improving symptoms.

\textsuperscript{\*} Corresponding author. Address: Department of Pediatrics, Tokushima Red Cross Hospital, 28-1, Shinbiraki, Chuden-cho, Komatsushima-shi, Tokushima 773-8502, Japan. Tel.: +81 8853 2 2555; fax: +81 8853 2 6350.
E-mail address: yoshihiro1973@me.pikara.ne.jp (Y. Toda).

Keywords: Secretin; Autism; BH\textsubscript{4}; HVA; 5HIAA

1. Introduction

A study has suggested that the dopamine/serotonin nervous systems are involved in the pathogenesis of autistic disorder [1]. In clinical practice, the efficacy of low-dose L-dihydroxyphenylalanine (L-DOPA), dopamine receptor blockers including major tranquilizers, and serotonin reuptake inhibitors has been reported [2], supporting the involvement of the dopamine/serotonin nervous systems.

Since, Horvath et al. reported the efficacy of secretin [3], secretin has recently been employed for treatment in the United States. However, no study has investigated the influence of secretin on the dopamine/serotonin nervous systems in children with autism. In this study, we investigated the effects of secretin (Secrepan\textsuperscript{w}®) on autism in 12 children, and measured the CSF levels of a metabolite of dopamine, homovanillic acid (HVA), a metabolite of serotonin, 5-hydroxyindole-3-acetic acid (5HIAA), and a coenzyme, 6R-5,6,7,8-tetrahydro-L-biopterin (BH\textsubscript{4}), of which the efficacy for autistic disorder has recently been reported [4], before and after administration of secretin to examine the relationship between changes in these parameters and improvement in symptoms.

2. Materials and methods

Prior to this clinical study, our protocol was approved by the Ethical Committee for Judging Clinical Studies on Medicines, Tokushima University Medical School Hospital. The subjects were 12 children who were diagnosed as having autistic disorder according to the DSM-IV, and were treated at the outpatient clinic of the Department of Pediatrics in Tokushima University. After the content and
The purpose of this clinical study were explained, written informed consent was obtained from all of the children’s parents (age range: 4–16 years, median age: 9 years, boy: girl = 8:4). The intellectual level was evaluated as mild (IQ: 50–69) in 4 children, moderate (IQ: 35–49) in 3 children, and severe (IQ: less than 35) in 5 children. IQ was determined using Tanaka-Binet’s formula.

In addition, blood examination (including blood cell examination, general biochemistry, chromosome, blood serum organic acid assay, thyroid hormone, lactate/pyruvate, immune globulin), urine metabolism screening examination, brain MRI, brain SPECT, electroencephalogram were all performed prior to the trial and patients with defect were excluded.

Among the 12 infants, only one (patient 6 in Figs. 1–3) had slight neonatal asphyxia (Apgar scores are uncertain), but there was no problem in the examination and coherence with autism was indistinct. In addition, two infants in this series were brothers (patients 8 and 10 in Figs. 1–3). None of the other infants had any problem in family history.

In a single-blind cross-over study, intravenous drip of secretin (Secrepan®) or physiological saline over 1 h was alternately administered at 4-week intervals (to a group in which physiological saline was intravenously infused 4 weeks after intravenous infusion of secretin (Group A, n = 5), and a group in which secretin was intravenously infused 4 weeks after intravenous infusion of physiological saline (Group B, n = 7)). The dose of secretin ranged from 8 to 12 units/kg. The blind method was employed for the children and their parents, and the contents were open to physicians. Behaviors were evaluated using the Autism Diagnostic Interview-Revised (ADI-R) before administration and 2, 4, 6, and 8 weeks after administration to investigate improvement in symptoms. To monitor the development of side effects, blood collection, general/biochemical blood examinations (liver/kidney function, amylase), and urinalysis were performed before administration and again 4 and 8 weeks after administration. Furthermore, the CSF levels of HVA, 5HIAA, and BH4 were measured by high performance liquid chromatography (HPLC) before and after the start of this 8-week trial (in the group administered secretin during the first half, cerebrospinal fluid was collected 8 weeks after administration, in those receiving secretin during the latter half, CSF was collected four weeks after administration).

Diagnostic Interview-Revised (ADI-R) before administration and 2, 4, 6, and 8 weeks after administration to investigate improvement in symptoms. To monitor the development of side effects, blood collection, general/biochemical blood examinations (liver/kidney function, amylase), and urinalysis were performed before administration and again 4 and 8 weeks after administration. Furthermore, the CSF levels of HVA, 5HIAA, and BH4 were measured by high performance liquid chromatography (HPLC) before and after the start of this 8-week trial (in the group administered secretin during the first half, cerebrospinal fluid was collected 8 weeks after administration, in those receiving secretin during the latter half, CSF was collected four weeks after administration).

---

Fig. 1. HVA levels in CSF before and after administration of secretin. The transverse axis shows the interval after administration of secretin (weeks). The longitudinal axis shows the HVA level. The children were divided into 2 groups, a group with improvement in the ADI-R score after administration of secretin (A) and a group without improvement (B). The patient numbers correspond to the same patients in Figs. 1–3. In all 7 children with improvement, the HVA level was increased. In 2 of the 5 children without improvement, the HVA level was increased. The control values were 91.9 ± 10.5 ng/ml (3–9 years) and 60.3 ± 12.7 ng/ml (10–15 years). The HVA level did not exceed the control value (2SD) in any child.

Fig. 2. 5HIAA levels in CSF before and after administration of secretin. In 5 of the 7 children with improvement, the 5HIAA level was increased. In 2 of the 5 children without improvement, the 5HIAA level was increased. The control values were 26.9 ± 5.5 ng/ml (3–9 years) and 20.6 ± 7.2 ng/ml (10–15 years). The 5HIAA level did not exceed the control value (2SD) in any child.

Fig. 3. BH4 levels in CSF before and after administration of secretin. In 5 of the 10 children, the BH4 level was increased. In all of the 5 children, improvement in the symptoms was achieved. In contrast, improvement was not achieved in the other 5 children without an increase in the BH4 level. The control value was 25.6 ± 12.1 pmol/ml (3–15 years). The BH4 level did not exceed the control value (2SD) in any child.
The CSF collected from almost all patients between 3:00 and 4:00 p.m. at the outpatient department, except case 3 at 11:00 a.m. We performed lumbar puncture with a spinal needle and promptly initiated light exclusion and cryopreservation after collection.

As control values, we measured the CSF levels of HVA, 5HIAA, and BH4 using CSF samples collected from children without psychiatric/neurological abnormalities from whom written informed consent was obtained after the purpose of this study was explained to their parents, among children in whom a CSF test was required under a tentative diagnosis of meningitis. We employed the measurements obtained in 10 children aged 3–9 years (median age: 15 years, boy:girl = 7:3) and 7 children aged 10–15 years (median age: 12 years, boy:girl = 5:2) in whom the general CSF test did not reveal any abnormalities; however, the BH4 level could be measured in only 8 children due to an insufficient volume of samples (median age: 8 years, boy:girl = 5:3).

3. Results

Improvement in the ADI-R score was achieved in 7 of the 12 children, but not in the remaining 5 children. In particular, the scores for qualities of reciprocal social interaction (improvement in 6 children; 5 of the 6 children aged less than 10 years, 1 of the 6 children aged 10 years or older) and communication/language (improvement in 4 children; all of the children aged less than 10 years) markedly improved; frequent eye contact, smile return, an increase in the number of words, and obedience to a person’s instructions were observed. The score for restricted and repetitive, stereotyped interests and behaviors improved in 2 children (all of the children aged less than 10 years); persistence to the same thing improved in 1 patient, and stereotyped behaviors in 1 patient. In addition, the scores for both qualities of reciprocal social interaction and communication/language improved in 2 children, both reciprocal social interaction and restricted and repetitive, stereotyped interests and behaviors improved in 1 patient, and all the items improved in 1 patient. In 2 of the 12 children (one aged less than 10 years, another aged 10 years or older), deterioration was observed; persistence to the same thing deteriorated in 1 child, and stereotyped behaviors in 1 patient.

According to an inquiry from the patients’ families, destructive behaviors such as temper, aggressiveness, and self-mutilation improved in 4 of the 12 children, although the ADI-R does not involve these factors. In 3 of the 12 children, hyperactivity and concentration improved. However, hyperactivity and temper exacerbated in 4 and 2 of the 12 children, respectively.

CSF could be collected before and after administration of secretin (twice) in 12 children.

The CSF level of HVA was increased in 9 of the 12 children (Fig. 1). In particular, in all children in whom administration of secretin improved the ADI-R score (7 of 12), the HVA level was increased (6 of these children were less than 10 years old). In 2 of the 5 children without improvement in the ADI-R score, the level was increased (all of these children were 10 years or older).

The CSF level of 5HIAA was increased in 7 of the 12 children (Fig. 2). In 5 of the 7 children with improvement in the ADI-R score, the 5HIAA level was increased (4 of these children were less than 10 years old) and in 2 of the 5 children without improvement, the level was increased (all of these children were 10 years or older).

In 2 children with deterioration of the score for restricted and repetitive, stereotyped interests and behaviors, a CSF test was performed (patients 2 and 3 in Figs. 1 and 2); the CSF levels of HVA and 5HIAA were increased. Of the controls, the CSF levels of HVA were 91.9 ± 10.5 ng/ml in the children aged 3–9 years and 60.3 ± 12.7 ng/ml in those aged 10–15 years. The CSF levels of 5HIAA were 26.9 ± 5.5 ng/ml in the children aged 3–9 years and 20.6 ± 7.2 ng/ml in those aged 10–15 years. The CSF levels of HVA and 5HIAA before and after administration of secretin did not exceed the control values (2SD) in any child with autism.

In 10 of the 12 children from whom CSF could be collected, the BH4 level could be measured before and after administration of secretin. The BH4 level was markedly increased in 5 children, whereas it was decreased or there was no change in 5 children (Fig. 3); however, improvement in the ADI-R score was achieved in all the children with the increase. In contrast, no child without an increase in the BH4 level achieved improvement in the score.

The CSF level of HVA increased in the 5 children in whom the BH4 level increased (4 of the 5 children were aged less than 10 years, 1 of the 5 children was aged 10 years or older) and in 2 of the 5 children without the BH4 level increase, the HVA level increased (all of these children were 10 years or older).

The CSF level of 5HIAA increased in 3 of the 5 children in whom the BH4 level increased, and in 2 of the 5 children without the BH4 level increase, the 5HIAA level increased (all of these children were 10 years or older).

In the control group, the CSF level of BH4 was 25.6 ± 12.1 pmol/ml (3–15 years). However, the CSF levels of BH4 before and after administration of secretin did not exceed the control value (2SD) in any child with autism.

The paired t-test was performed on the concentration changes after secretin administration for HVA, 5HIAA, and BH4. There was a significant difference in the concentration rise of HVA in the whole (P = 0.04). On the other hand, there was no significant difference in the whole concentration rise in 5HIAA. Moreover, there was no significant difference in the children with ADI-R score improvement (P = 0.18) in 5HIAA. Although there was no significant difference on the whole in BH4 (P = 0.22), there was...
a significant difference in the children with ADI-R score improvement \((P=0.04)\).

Neither hematology nor urinalysis showed any abnormalities before or after administration of secretin.

4. Discussion

The main symptoms of autism include disorders in reciprocal social interaction, communication disorders, and imagination disorders (localized interests, persistence, and resistance to changes). In the latter half of the 1960s, the conventional hypothesis that psychogenic factors are involved in autism was ruled out, and it has been indicated that biological abnormalities in the brain are etiologically involved; however, the pathogenesis/etiology of this disorder remain to be clarified in many respects.

Segawa [1] has assumed that reduction of serotonin/dopamine nervous system activity early in the morning is involved in the pathogenesis of autism. Briefly, according to his hypothesis, disorders in the serotonin system early in the morning cause developmental disorders in the sleep/waking rhythm and disorders in reciprocal social interaction (disorder in instinctive behaviors and adaptation disorder for environmental changes), and induce differentiation of cerebral hemisphere function and functional differentiation disorder with respect to cerebral sites. In addition, the dopamine nervous system causes hyperactivity, stereotyped behaviors, and panic via secondary receptor hypersensitivity, inducing frontal lobe dysfunction. He has reported that drug therapy with 5-hydroxytryptophan (5-HTP) and an extremely low dose of L-DOPA improved disorders in the sleep/waking rhythm and emotional/behavioral abnormalities, which are characteristic of autism, in many patients [1].

Naruse et al. [4] speculated that amine synthesis is reduced in children with autism based on the finding that the synthesis of serotonin/catecholamine is disordered in children with phenylketonuria and autistic tendency, and administered R-tetrahydrobiopterin (R-THBP), which acts as the coenzyme of tyrosine hydroxylase/tryptophan hydroxylase important in the process of dopamine/serotonin synthesis. Improvement in human relationships/social interaction and language was achieved in a high percentage of the children; however, in another clinical trial involving a larger number of hospitals, significant improvement was not achieved, and in Japan, R-THBP is approved only for bioppterin deficiency. However, thereafter, Fernell et al. [5] and Komori et al. [6] administered R-THBP to children with autism in whom the CSF level of R-THBP was relatively low, and improvement in social interaction/language was achieved in all children, suggesting the efficacy of this agent for a subgroup of autism.

In this study, we investigated the effects of secretin on infantile autism, and this agent improved the score for reciprocal social interaction in 6 children and that for communication/language in 4 children. In addition, the CSF level of BH₄ after administration of secretin was increased compared to the pretreatment value in all children with improvement. In contrast, no child without an increase in the BH₄ level achieved improvement in the score. In many children with autism, the CSF levels of HVA were increased after administration. This suggests that secretin activates metabolic turnover of dopamine in the central nervous system via an increase in the BH₄ level.

The ADI-R scores for qualities of reciprocal social interaction and communication/language more markedly improved in the children aged less than 10 years. With respect to this finding, Segawa administered 5-HTP and L-DOPA to children with autism, and reported that the treatment response was more marked at a lower age; in particular, concerning the serotonin system, there was no improvement in any child treated with 5-HTP at the age of 10 years or older [1], suggesting that the effects of drug therapy via the dopamine/serotonin nervous systems depend on age.

With regard to the BH₄ level, the number of control children was small, and in this study, it was difficult to establish control values with respect to age. However, according to Blau et al. [7], the control values of BH₄ were 19.1–54.1 pmol/ml in children aged 5–10 years and 19.0–44.3 pmol/ml in those aged 10–16 years. In reference to these values, the BH₄ level was low in 1 child (patient 3) before administration of secretin. In this child, the BH₄ level was rapidly increased after administration of secretin, with improvement in the symptoms (deterioration in some parameters, as described below). Therefore, the condition may resemble BH₄ deficiency, and administration of R-THBP may be effective.

With respect to age, the children with an increase in the BH₄ level, excluding the above child, were less than 10 years old. The reason why there was no increase in the BH₄ level in any child aged 10 years or older is unclear; however, sensitivity may be reduced in an age-dependent manner. According to Blau et al. [7], the BH₄ level was high in infants aged less than 1 year, especially those aged less than 6 months, but then decreased with age. This suggests the importance of BH₄ in the developmental/establishment process of the central nervous system in the initial stage; the biosynthesis of BH₄ may be inhibited to some degree after this period.

Among the ADI-R parameters, the score for restricted repetitive behaviors and interests deteriorated in 2 children. In all of these children, a CSF test was performed, and the HVA and 5HIAA levels were increased. The BH₄ level could be measured in 1 (patient 3) of these 2 children, and a marked increase was observed. Administration of L-DOPA at 0.5 mg/kg/day to children with autism was effective; however, no response or deterioration of the symptoms was observed at a higher dose. Therefore, receptor hypersensitivity related to reduction of dopamine activity may be present in patients with autism [1]. In these 2 children,
administration of secretin markedly increased dopamine activity, further stimulating hypersensitive dopamine receptors and deteriorating the symptoms.

Since, Horvath et al. reported the efficacy of secretin in 1998 [3], clinical trials of secretin for autism have been conducted throughout the world; however, many studies considered the effects doubtful [8,9]. However, if autism is a syndrome associated with various etiological factors, it may be natural that there are responders and non-responders; we cannot always rule out the efficacy based on the finding that statistical analysis in a large number of patients does not show any significant difference. In contrast, the etiology may be clarified by investigating these patients and dividing them into new subgroups.

Secretin is present in the cerebral cortex, thalamus, hypothalamus, and brain stem. Its receptors have been found in these areas, and are considered to act as a neurotransmitter [10]. Secretin is also known to activate tyrosine hydroxylase in the maxillary ganglion [11]. However, the results of this study suggest that secretin activates tyrosine hydroxylase via BH$_4$, influencing the dopamine nervous systems. In the future, the detailed mechanism should be further investigated.

References


