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Sulfamethoxazole-trimethoprim double-blind, placebo-controlled, crossover trial in Machado-Joseph disease: sulfamethoxazole-trimethoprim increases cerebrospinal fluid level of biopterin

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Summary. We performed a double-blind, placebo-controlled, crossover trial of sulfamethoxazole-trimethoprim (S-T) in 8 patients with Machado-Joseph disease (MJD), and measured the blood and cerebrospinal fluid levels of biopterins, biogenic amines or metabolites, and folate. The clinical results were as follows; mild improvements of hyperreflexia of knee jerks and of rigo-spasticity of the legs during S-T treatment period. In addition, S-T significantly reduced the times of 8 motor activities on the timed tests. The biochemical results showed that basal levels of all biopterins and homovanillic acid in the cerebrospinal fluid (CSF) were reduced to less than half the levels of those of controls with other neurological diseases. After S-T treatment, total and oxidized form of biopterins in the CSF increased significantly. Therefore, S-T may be effective to neurologic deficits through its mechanism of increasing the level of brain biopterins.

Keywords: Sulfamethoxazole-trimethoprim, Machado-Joseph disease, dihydrofolate reductase, dihydropteridine reductase, tetrahydrobiopterin.

Introduction

Machado-Joseph disease (MJD) is a dominantly inherited spinocerebellar degeneration that had been reported exclusively from U.S.A. (Nakano et al., 1972; Woods and Schaumburg, 1972; Rosenberg, 1991) and Portugal (Coutinho and Andrade, 1978) until 1983, when we first reported a non-Portuguese family of pathologically proven Joseph disease from Japan (Sakai et al., 1983). Thereafter, many cases were reported from various countries. Even though biochemical and genetic studies were reported (Rosenberg et al., 1979; Grossman et al., 1987; Takiyama et al., 1993; Kawaguchi et al., 1994), the pathogenesis remains to be elucidated. Woods and Schaumburg (1972) mentioned a partial relief of symptoms by antiparkinsonian drugs in their original

cases. Interestingly, Mello and Abbott (1988) incidentally found an effectiveness of sulfamethoxazole-trimethoprim (S-T), an antimicrobial combination which was prescribed for the treatment of dysuria, on neurologic dysfunction in a patient with MJD. However, the mechanism by which S-T exerts its pharmacological actions on the central nervous system (CNS) was not delineated.

The purposes of the present study are to investigate whether or not S-T is effective in alleviating the neurological symptoms and signs in MJD, and then to test the following hypothesis concerning the mechanism how S-T exerts its pharmacological actions on the neurological deficits; before administration of S-T, tetrahydrobiopterin (BH4) is decreased in MJD brains. On the other hand, when S-T is provided, trimethoprim, an inhibitor of dihydrofolate reductase (DHFR), acts not only on the DHFR, but also on the CNS enzyme, dihydropteridine reductase (DHPR), for both enzymes have homology. This, finally, results in an enhanced turnover rate of brain BH4 with the increased levels of neurotransmitters which will lead to the neurological improvements.

Subjects and methods

Subjects

There were eight patients enrolled into the double-blind trial, who were diagnosed with pathologically proven, "clinically definite" or "probable" MJD according to our previously published criteria (Sakai, 1989; Sakai et al., 1993) (Table 1). Two patients (case nos. 1, 2, in Table 1) were from a pathologically proven family, two patients (case nos. 4, 7) from a "clinically definite" family, and the rest were from four unrelated "probable" families. Except for case 8, at least one member from each family was genetically checked, and trinucleotide (CAG) repeat was confirmed on the 14th chromosome (Kawaguchi et al., 1994). Five patients received medication acting on CNS to alleviate dystonia and

Table 1. Demographic data of 8 patients with Machado-Joseph disease

Case no.	1	2	3	4	5	6	7	8
Age/Sex	31/M	33/M	38/M	46/F	50/M	54/M	55/M	60/M
Duration	15	13	17	15	5	10	21	11 years
Neurol. Exa.								
Extrapyr.	++	++	++	++	−	±	+	−
Pyramidal	++	++	++	++	+	+	++	+
Cerebell.	−	−	+	+	+	++	++	++
Amyotrophy	++	++	++	++	−	−	++	+
Type[a]	I	I	I	I	II	II	II	II
Treatment[b]	(+)	(+)	(+)	(+)	(−)	(+)	(−)	(−)
① THP (mg)	4	2	8	4		2		
② Levodopa + Carbidopa (mg)			250 +25	250 +25				

Abbreviations: *Extrapyr.* extrapyramidal signs, *Cerebell.* cerebellar signs, ++ marked, + mild, ± minimal, − absent, *THP* trihexyphenidyl hydrochloride. [a] Type: each patient was categorized according to our previous reports[14,15]. [b] Treatment: indicates medication influencing the central nervous sytem, and does not include other medication

parkinsonism, and the rest took no medication affecting the nervous system. Treatment regimen, whether active in the CNS or not, remained uninterrupted throughout the double-blind, crossover trial period. Before entry into the trial, electrocardiography and blood examination (hematology, hepatic and renal functions) were performed to exclude the patients with abnormal results. After all patients were hospitalized, they gave informed consent which was approved by the hospital internal review board.

Eight patients were selected as age- and activities of daily life (ADL)-matched diseased controls and informed consent was obtained from all patients. The diagnoses of diseased controls were as following; four patients with Becker muscular dystrophy, and one each with Duchenne muscular dystrophy, myotonic muscular dystrophy, facioscapulohumeral muscular dystrophy, and Sjögren syndrome.

Treatment

S-T tablets, containing 400 mg and 80 mg respectively, were crushed and converted into capsule form by the pharmacy of National Chikugo Hospital, Chikugo City, Japan. S-T and placebo (lactose) capsules were of equal color, size, and weight and identified as drugs A and B by a randomized process. Since two patients had difficulty taking foods and liquid, a blinded granule of placebo as well as S-T, 1 gram containing sulfamethoxazole 400 mg and trimethoprim 80 mg, was administered two times a day to these patients, using a nasogastric tube. All patients were treated with S-T and placebo for 4 weeks each according to a randomized, double-blind, crossover study design with a washout period of 2 weeks between the first and second treatment periods. S-T and placebo capsules or blinded granules were given after breakfast and dinner to each patient with a daily dose of sulfamethoxazole 800 mg and trimethoprim 160 mg. Compliance was checked each day by counting capsules or blinded granules.

Assessment

A series of six questions, neurological examinations, and timed testings were carried out once a week in all the patients as follows:

(1) *Questionnaire*: Six questions of subjective feelings related to speech, choking, dexterity, stationary balance, ability to walk, and overall sense of general well-being. A 5-point nominal scale ranging from ++ to −− was used. The patient was asked to indicate 0 in case of no change, + or − in case of mild improvement or deterioration, and ++ or −− in case of significant improvement or deterioration, respectively. For each treatment period, the answer of the patient was based on a subjective feeling compared with that immediately before the start of the treatment period.

(2) *Neurological examinations*: A standard grading system was used by one examiner (T.S.) to prevent interrater variability. The following tests were performed: severity of dystonia or athetosis, spasticity or rigidity of the limbs, bradykinesia in finger-tapping, deep tendon reflexes (DTRs), dysarthria and cerebellar testing such as finger-to-nose, heel-to-knee tests and diadochokinesis.

(3) *Timed testing*: To detect a mild but significant change of neurological deficits, the below-listed motor activities were measured with a stop-watch of 1/60 accuracy. Two trials of each task were performed and recorded and the mean was used in the statistical analyses.

1. finger-to-nose test (10 times, bilaterally). 2. finger-tapping test (10 times, bilaterally). 3. hand diadochokinesis (10 times, bilaterally). 4. heel-to-knee tapping test (10 times, bilaterally). 5. oropharyngeal repetition test (the seconds taken to say one-syllable "Pa" and two-syllable "Pata" repetitions 10 times each). 6. slotted can (the seconds taken to place 5 coins into a slotted can with a dominant hand). 7. dial telephone (the seconds to dial a given seven-digit number, 5-2-7-1-9-4-8, on a rotatory dial telephone with a dominant hand). 8. walking

time (the seconds to walk 10 meters if the patient is ambulatory or 3.5 meters both ways holding parallel bars if not ambulatory).

Blood studies [complete blood count (CBC), total serum protein and its electrophoretic pattern, bilirubin, alkaline phosphatase, glutamic-oxaloacetic (GOT) and glutamic-pyruvic transaminases (GPT), blood urea nitrogen (BUN), creatine, creatinine, creatine kinase (CK), lactic dehydrogenase (LDH), cholesterol, triglyceride, and electrolytes] and urinalysis were performed before and after each 4-week period of treatment.

Withdrawal

Patients were allowed to withdraw from the trial at any time, whether of their own free will or due to adverse effects of the drugs.

Lumbar puncture

Lumbar puncture was performed between 8:30 and 11:00 am before the start of and again on the final day of the 4-week S-T treatment and placebo periods in the 7 patients (case nos. 1–4, 6–8 in Table 1) who fasted overnight. A 6-ml aliquot of cerebrospinal fluid (CSF) was drawn into a sunbeam-cut tube on ice for the assay of amines, biopterins, folate and other biochemical entities. An additional 2 ml of CSF was used for obtaining cell count and for measuring glucose and protein.

Biopterin assay, biogenic amine and folate measurements

The biopterin assay was carried out according to the method of Fukushima and Nixon (1980). Their method is based upon the following principle. Using iodine in 0.1 N HCl, 7,8-dihydrobiopterin (BH2) and tetrahydrobiopterin (BH4) are effectively oxidized to biopterin, while, with the use of 0.1 N NaOH, 90% of BH2 is recovered as biopterin but 80% of BH4 is converted to pterin. 0.4 ml CSF or 0.3 ml serum was collected into a tube containing iodine in 0.1 N HCl or 0.1 N NaOH, shaken, and stored at −80°C until assay. After each sample was subjected to partial purification on a Dowex 50 column, it was injected into a high performance liquid chromatograph (Komori et al., 1994). The sample after acid oxidation represents the total biopterin (total B; BH4, quinonoid dihydrobiopterin, BH2, and biopterin), and the sample after alkaline oxidation represents the oxidized form of biopterin (oxidized B; BH2 and oxidized biopterin). Thus, the difference between the total B and oxidized B is the reduced form of biopterin (reduced B; BH4 and quinonoid BH2). Catecholamines, serotonin, homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (HIAA) were measured, using high performance liquid chromatography with an electrochemical detector (Yokoo et al., 1991). Serum and CSF folate were measured by a commercial radioassay kit (Ciba-Corning).

Statistical analyses

A statistical comparison between the S-T and placebo periods was performed with Wilcoxon signed-rank test for subjective data and objective examinations in patients who completed the trial.

Before the treatments, the comparative basal levels of biopterins, biogenic amines or their metabolites, and folate between MJD and age- and ADL-matched diseased controls were analyzed using the F-test and Student's t-test. The levels of biopterins, biogenic amines or metabolites, and folate were compared by Wilcoxon signed-rank test between S-T treatment and placebo periods in MJD patients. A p value less than 0.05 at two-tailed tests was considered to be statistically significant. The correlation between variables was analyzed using Spearman's rank correlation coefficient.

Results

All eight patients completed the trial without any adverse effects.

1. Clinical effects of S-T

A. Questionnaire: There was no significant change of subjective feelings between placebo and S-T treatment periods.
B. Neurological examinations: A statistical analysis of group comparison between S-T and placebo periods revealed a significant decrease of hyperreflexia of knee jerks at the 4th week (mean ± S.D. = 3.0 ± 1.1 during placebo vs. 2.5 ± 0.6 during S-T period; $p < 0.05$ at two-tailed tests) and a mild improvement of rigidity-spasticity of the legs at the 3rd week (2.2 ± 0.8 during placebo vs. 1.8 ± 0.9 during S-T period; $p < 0.10$). There was no significant change of dystonia, bradykinesia, dysarthria or cerebellar tests.
C. Timed testing (Table 2): A walking time was significantly improved at the 1st and 4th weeks of S-T period (both $p < 0.05$ at two-tailed tests) and the mean values were reduced to half those of placebo period. Additionally, the time to take the following motor activities was significantly reduced; finger-to-nose test by right hand at 2nd week ($p < 0.05$), finger-tapping by left hand at 4th week ($p < 0.05$), diadochokinesis by both hands (right hand at 3rd and 4th weeks, $p < 0.05$ and $p < 0.01$, respectively; left hand at 3rd week, $p < 0.05$), heel-to-knee tapping test by both legs at 4th week (both $p < 0.05$), two-syllable "Pata" repetitions at 4th week ($p < 0.05$), and slotted can and dial telephone at 4th week ($p < 0.01$ and $p < 0.05$, respectively).

2. Biochemical results

A. Before sulfamethoxazole-trimethoprim treatment

Cerebrospinal fluid level (Table 3): There was no significant difference in age between 7 patients with MJD and 8 patients with other neurological diseases (O.N.D.). Total B, oxidized B and reduced B were markedly decreased in MJD to 45%, 47%, and 44% of levels in O.N.D., respectively. The CSF level of homovanillic acid (HVA) was decreased in MJD to less than half the level of O.N.D. The CSF level of folate was mildly decreased in MJD, but the difference was not significant ($p < 0.10$).
Blood level: Neither biopterins, plasma norepinephrine, serotonin, nor folate were significantly different between MJD and O.N.D. (data not shown here).

B. After sulfamethoxazole-trimethoprim treatment

Cerebrospinal fluid level (Table 4): CSF total B was significantly increased during the S-T period compared with that during the placebo period ($p < 0.05$, two-tailed tests: Fig. 1A). CSF oxidized B was increased during the S-T period ($p < 0.02$: Fig. 1B). The CSF reduced B was not significantly increased from placebo to S-T periods (Fig. 1C). Though the mean values of both CSF HVA and HIAA were increased during S-T period, the increments were not signifi-

Table 2. Results of timed tests in 8 patients with Machado-Joseph disease

		1	2	3	4 week
① finger-to-nose	(R)		19 ± 7 vs. 16 ± 5*		
	(L)				
② finger-tapping	(R)				
	(L)				13 ± 9 vs. 9 ± 3*
③ hand diadocho-kniesis	(R)			21 ± 11 vs. 18 ± 9*	22 ± 10 vs. 18 ± 8**
	(L)			22 ± 11 vs. 18 ± 8*	
④ heel-to-knee tapping	(R)				7 ± 3 vs. 5 ± 3*
	(L)				9 ± 9 vs. 6 ± 4*
⑤ oropharyngeal repetition	"Pa"				
	"Pata"				30 ± 49 vs. 8 ± 3*
⑥ slotted can					103 ± 164 vs. 38 ± 25**
⑦ dial telephone					107 ± 164 vs. 72 ± 110*
⑧ walking time		177 ± 191 vs. 92 ± 92*			165 ± 168 vs. 84 ± 96*

Abbreviation: (R), (L) indicate right-sided, left-sided, respectively. Notes: The values in table imply the mean ± standard deviation, expressed in second. A vs. B indicates that A is the time taken to perform the task during placebo period and B is the time during S-T period. *,** imply p values less than 0.05, and 0.01 at two-tailed, respectively which are analyzed by Wilcoxon signed-rank test. Vacant space indicates that the given task is statistically not significant between placebo and S-T periods

Table 3. Basal CSF levels of biopterins, amine metabolites, and folate

	Other neurological disease (O.N.D.)	Machado-Joseph Disease (MJD)	p value
Number	n = 8	n = 7	
Age	48.1 ± 14.1	45.7 ± 12.1	NS
Sex	M:F = 6:2	M:F = 6:1	
TB	3.88 ± 1.10	1.74 ± 0.67	$p < .001$
OB	1.80 ± 0.42	0.84 ± 0.19	$p < .001$
RB	2.08 ± 0.76	0.91 ± 0.50	$p < .005$
R/T B	0.52 ± 0.08	0.50 ± 0.09	NS
HVA	45.8 ± 15.6	21.0 ± 13.4	$p < .01$
HIAA	25.7 ± 13.8	40.0 ± 11.7	NS
HIAA/HVA	0.57 ± 0.21	2.47 ± 1.15	$p < .01$
Folate	11.2 ± 2.42	9.06 ± 1.94	NS

Abbreviations: *NS* not significant, *TB* total biopterin, *OB* oxidized form of biopterin, *RB* reduced form of biopterin, *R/T B* a ratio of reduced form to total biopterin, *HVA* homovanillic acid, *HIAA* 5-hydroxyindoleacetic acid, *HIAA/HVA* a ratio of HIAA to HVA

Table 4. Changes of CSF metabolites by sulfamethoxazole-trimethoprim

	TB (ng/ml)		OB (ng/ml)		RB (ng/ml)		R/T B	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Placebo	1.46	0.47	0.64	0.17	0.81	0.41	0.54	0.14
S-T	1.94	0.62	1.10	0.33	0.84	0.51	0.42	0.17
p value	<.05		<.02		NS		NS	
	HVA (ng/ml)		HIAA (ng/ml)		HIAA/HVA		Folate (ng/ml)	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Placebo	12.9	8.77	35.7	21.6	3.03	1.02	8.44	1.73
S-T	19.6	13.9	36.5	12.7	2.35	0.90	8.16	2.34
p value	NS		NS		<.05		NS	

Abbreviations: *TB* total biopterin, *OB* oxidized form of biopterin, *RB* reduced form of biopterin, *R/T B* a ratio of RB to TB, *HVA* homovanillic acid, *HIAA* 5-hydroxyindoleacetic acid, *HIAA/HVA* a ratio of HIAA to HVA, *S-T* sulfamethoxazole-trimethoprim, *NS* not significant

cant. The ratio of HIAA to HVA was significantly decreased during the S-T period.

Blood level: Serum total, oxidized, and reduced Bs, the ratio of reduced B to total B, and plasma norepinephrine were not significantly different between the placebo and S-T period. On the other hand, serum folate level significantly fell from 8.3 ± 7.2 (mean ± S.D.) ng/ml during the placebo period to 6.1 ± 5.3 ng/ml during the S-T period ($p < 0.01$).

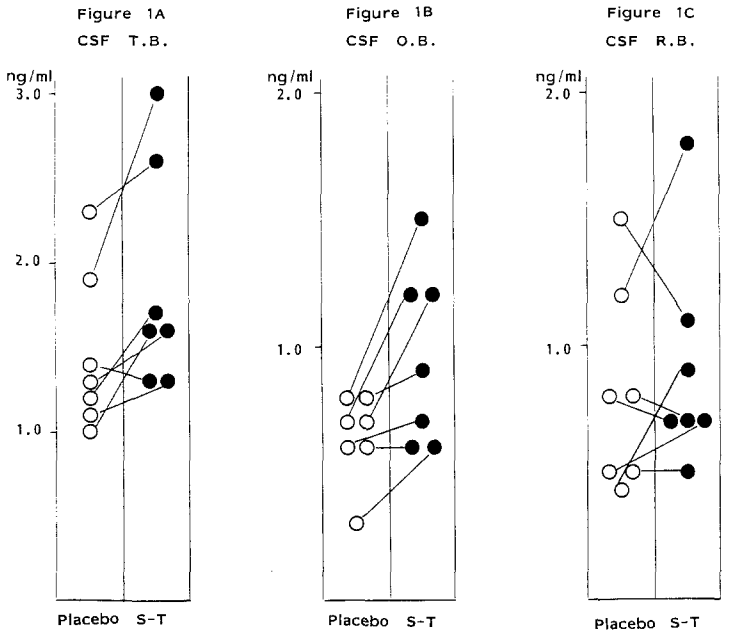

Fig. 1. Changes in CSF biopterins from placebo to sulfamethoxazole-trimethoprim (S-T) periods in patients with Machado-Joseph disease (MJD). **A** CSF level of total biopterin (TB) was significantly increased in MJD from placebo period to S-T period. **B** CSF level of oxidized form of biopterin (OB) was significantly increased in MJD from placebo period to S-T period. **C** Though the mean value of CSF level of reduced form of biopterin (RB) was increased from placebo period to S-T period, the difference was not significant

C. Correlations between CSF metabolite variables

Of note are the changes in the correlative coefficient between reduced B and HVA from $r = +0.32$ (not significant) during placebo to $+0.89$ ($p < 0.01$) during the S-T period, and between reduced B and HIAA from $+0.26$ (not significant) during placebo to $+0.86$ ($p < 0.05$) during the S-T period.

Discussion

Mello and Abbott unexpectedly found that S-T could lessen spasticity of the legs and improve gait disturbance in a patient with MJD, and they speculated that an effect of one or both components of S-T on neurotransmitter function might have occurred (1988). But the exact mechanism remained to be elucidated. Since then, there have been 4 similar reports (Sangla et al., 1990; Takiyama et al., 1990; Spinella and Sheridan, 1992; Noro and Minami, 1992). Sangla et al. described improvements of spasticity and dystonia in a patient with MJD (1990). Takiyama et al. reported a drastic change of muscle tonus of the arm from marked rigidity to normal during S-T treatment in one of three patients with MJD (1990). In the present trial, S-T was effective in improving the hyperreflexia of knee jerks and rigo-spasticity of the legs. Additionally, the timed tests revealed that 8 motor activities were significantly

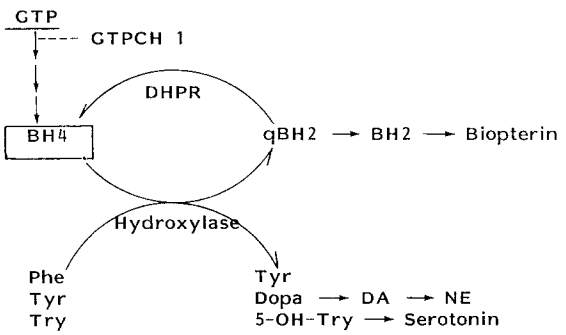

Fig. 2. Metabolism of tetrahydrobiopterin (BH4). Abbreviations: *GTP* guanosine triphosphate, *GTPCH I* GTP cyclohydrolase I, *BH4* tetrahydrobiopterin, *qBH2* quinonoid dihydrobiopterin, *BH2* 7,8-dihydrobiopterin, *DHPR* dihydropteridine reductase, *Phe* phenylalanine, *Tyr* tyrosine, *Try* tryptophan, *DA* dopamine, *NE* norepinephrine, *5-OH-Try* 5-hydroxytryptophan

improved. The present results, i.e., a significant improvement of hyperreflexia and a trend of mild improvement of rigidity-spasticity may suggest that improvements on timed tests should be greatly influenced by amelioration of extrapyramidal signs in addition to pyramidal signs. The finding that the walking time on timed tests was significantly reduced seems to suggest the possibility that S-T may improve cerebellar ataxic gait. Interestingly, depression was remarkably improved in a patient, aged 55 (Case 7 in Table 1).

As to the mechanism of S-T on the neurological dysfunctions in MJD, we made a hypothesis which was deduced from 4 facts. First, there is no known human enzyme that is influenced by sulfamethoxazole (Finch and Snyder, 1990). Second, there exists an enzyme, DHFR, in humans as well as in bacteria the activity of which is selectively inhibited by trimethoprim (Burchall, 1973). Third, DHFR is reported to have a homology with DHPR (Dahl et al., 1987; Lockyer et al., 1987; Ratnam et al., 1989) which plays an important role in the recycling synthesis of BH4. Thus, the possibility emerges that trimethoprim inhibits not only CNS DHFR, but also CNS DHPR. If so, inhibition of DHPR activity will lead to a decreased turnover of the BH4 recycling pathway, which should, in turn, result in a feedback stimulation to de novo synthesis of BH4 from guanosine triphosphate (GTP), as seen in children with an inborn error of DHPR deficiency (Blau et al., 1992) (Fig. 2). Fourth, there were many reports confirming the markedly decreased level of HVA in the CSF of MJD (Sakai et al., 1983; Rosenberg, 1991). Taking these into account, we hypothesized that, before administration of S-T, BH4 content is decreased in MJD brains with the resultant decrease in the activities of phenylalanine-, tyrosine, and tryptophan hydroxylases. This leads to a decreased level of dopamine, norepinephrine, and serotonin. On the other hand, when S-T is given, trimethoprim will increase the CNS turnover rate of BH4. The result will be enhanced hydroxylation of phenylalanine, tyrosine, and tryptophan.

In the present study, we demonstrated that, before the treatments, basal CSF levels of various biopterins and of HVA were remarkably decreased in 7 patients with MJD (biopterin levels were less than half those in other neuro-

logical disease controls). There are four possible explanations for how CSF biopterins are reduced in MJD. First, degenerations of nigrostriatal dopaminergic neurons and possibly of BH4-containing non-dopaminergic neurons may be the cause because it is well-known that total B is concentrated in the striatum, especially in the dopaminergic nerve terminals (Sawada et al., 1987). Since one of the cardinal pathological characteristics in MJD is the degeneration of the substantia nigra (Rosenberg, 1991), this could be the case. Second, there might be an immaturity in the development of nigrostriatal dopaminergic neurons and other systems. However, such a pathological abnormality was never described in MJD brains (Woods and Schaumburg, 1972; Sakai et al., 1983; Rosenberg, 1991). Third, an inborn error of biopterin metabolism could be responsible. There are three inherited diseases of BH4 metabolism, i.e., GTP cyclohydrolase I (Niederwieser et al., 1984), 6-pyruvoyltetrahydropterin synthase (Shintaku et al., 1988), and DHPR deficiencies (Kaufman et al., 1975). Among these metabolic diseases, the first two are associated with a reduction of both CSF total and reduced Bs, while the third is associated with increments of total and oxidized Bs, but no change or a decrement of reduced B. But, the following facts argue against the possibility of MJD as an inherited metabolic disease of BH4. First, MJD is an autosomal dominant disease, while these inherited diseases of BH4 metabolism are supposed to be autosomal recessive disorders. Second, the blood levels of biopterins were shown to be normal in the present study, and there is no case report of BH4 metabolic disease in which a BH4 deficiency is limited only to the CNS. These facts throw a doubt on such a possibility. There is finally the possibility that medication may influence the CSF biopterin. But, the finding that CSF total B was likewise reduced in the non-medication group (data not shown here) renders this as a remote possibility. Taking the aforementioned together, it is plausible that a marked reduction of CSF biopterins may reflect a degeneration of nigrostriatal dopaminergic neurons and perhaps of BH4-containing extranigral non-dopaminergic neurons. The study that total B and GTP cyclohydrolase I activity were decreased in the striatum of the parkinsonian brains (Nagatsu et al., 1986) seems to support the above conclusion.

After S-T administration, the present study showed that the treatment produced increases of total and oxidized Bs, a decrease in the ratio of HIAA/HVA, and no change of reduced B in the CSF. On the other hand, blood measurements showed no changes of the biopterins, and a decrease in serum folate. Increases of CSF total and oxidized Bs, and no change of CSF reduced B are typically recognized in DHPR deficiency or inhibition. Thus, it is rational to consider that trimethoprim would have inhibited the activity of the CNS DHPR though we have no direct evidence for it and that it would have affected the peripheral tissue DHFR, leading to a decrease of serum folate level.

The CSF biopterin levels have been reported for some neurological diseases. Kay et al. reported that the CSF total B was decreased in Alzheimer disease to 71% of that in normal controls (1986). It was reported to be reduced in Parkinson disease to 73% of that in ventricular CSF from neurological controls (Furukawa et al., 1991), and, in another study, to 37% of that

in non-neurological patients (Fujishiro et al., 1990). Fink et al. reported that 4 dystonic patients with marked diurnal fluctuations had a remarkable reduction in CSF total B (1988). There has been no study on CSF biopterins in MJD to date.

BH4 recently attracts much attention not only from laboratory scientists but from clinicians, for it is well-known that BH4 is the essential coenzyme for three aromatic amino acid hydroxylases. In addition, it is discovered that BH4 has a critical regulatory role in the modulation of release of dopamine (Koshimura et al., 1990), serotonin (Mataga et al., 1991), acetylcholine (Ohue et al., 1993), glutamate and others. Moreover, several lines of evidence suggests that BH4 is essential as a cofactor for the activity of both constitutive nitric oxide synthase (NOS) in the brain and vascular endothelium and inducible NOS in the macrophages (Nunokawa et al., 1992; Moncada and Higgs, 1993). An immunohistochemical study revealed that the density of NOS was the highest in the cerebellum (Bredt et al., 1990). Therefore, it may be rational to consider that a BH4 deficiency of the brain would induce not only deficiencies or deranged release of various neurotransmitters, but also an attenuated generation of nitric oxide, a neuronal messenger molecule, in the cerebellar neurons which might be closely related to cerebellar signs seen in MJD.

The present study seems to provide two perspectives on the treatment of MJD. One is to decide the issue of whether or not trimethoprim as a monotherapy can improve neurological symptoms and signs in MJD. Another is that BH4 emerges as a potent therapeutic strategy in MJD. These trials are in progress in our hospital. Likewise, these trials may be worthy of being performed in other spinocerebellar degenerations, for instance, SCA1 and SCA2. Desaiah et al. reported that the NOS activity was significantly decreased in SCA1 cerebellum (1994), which may justify a therapeutic trial of BH4.

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