VITAMIN B₁₂ LEVELS OF CEREBROSPINAL FLUID IN PATIENTS WITH A VARIETY OF NEUROLOGICAL DISORDERS

Hirokuni TAGUCHI,¹ Hiroshi SANADA,¹ Koichi HARA,²
Isao MIYOSHI,² and Kiyoshi HIRAKI³

¹Central Laboratories, Okayama University Medical School, Okayama 700, Japan

²Department of Internal Medicine, Okayama University Medical School,
Okayama 700, Japan

³Kochi Medical School, Kochi 780, Japan

(Received February 21, 1977)

Summary The vitamin B₁₂ levels of cerebrospinal fluid were assayed microbiologically (*Lactobacillus leichmannii* method) using samples from 44 patients with various neurological disorders, 4 patients with megaloblastic anemia and 34 controls. Twenty-seven controls that did not receive vitamin B₁₂ showed a mean cerebrospinal fluid vitamin B₁₂ level of 21.5 pg/ml (range: 0-60). No decrease in cerebrospinal fluid vitamin B₁₂ level was seen in patients with subacute myelo-optico-neuropathy (SMON). High levels of cerebrospinal fluid vitamin B₁₂ were observed only in the patients receiving long term administration of the vitamin. Intrathecal administration of vitamin B₁₂ caused only a slight increase in serum vitamin B₁₂ level after four hours. The existence of a blood brain barrier for vitamin B₁₂ was suggested.

The role of vitamin B₁₂ (B₁₂) in the central nervous system has not yet been fully elucidated. Subacute combined degeneration of the cord (SACD) in pernicious anemia is the only occasion in which B₁₂ is associated with the cause of neurological symptoms (1). Subacute myelo-optico-neuropathy (SMON) (2, 3) have been attracting attention since early reports because of the similarity of the clinical features with those of SACD (4). Many attempts to treat SMON with B₁₂ resulted in failure and no decrease of serum B₁₂ levels have been reported in SMON (5). Nevertheless, Ukyo (6) reported that intrathecal B₁₂ administration produced some improvement in neurological manifestations in SMON. Although many papers have reported B₁₂ levels in cerebrospinal fluid (CSF) (7-14),

¹ 田口博國, 真田 浩, ² 原 功一, 三好勇夫, ³ 平木 潔

Abbreviations used: B₁₂, vitamin B₁₂; SACD, subacute combined degeneration of the cord; SMON, subacute myelo-optico-neuropathy; CSF, cerebrospinal fluid.

little is known about the transport of B_{12} from the blood to the CSF (15) and *vice versa* (6, 16). The purposes of this paper are to report CSF B_{12} levels in various neurological diseases especially in SMON and to see whether blood B_{12} is easily transported to and from the CSF.

MATERIALS AND METHODS

Serum and CSF B_{12} levels were assayed microbiologically using *Lactobacillus leichmannii* as a test organism (17). Three to 5 ml of CSF was used for KCN extraction and the final volume was adjusted to 10 ml instead of 25 ml for blood B_{12} assay. This diminution of dilution rate enabled CSF B_{12} levels to be assayed in the same range of standard curve as those of blood. CSF B_{12} levels were assayed for 34 controls and 44 patients with various neurological disorders, including 23 cases of SMON as listed in Table 1. Controls were subjects with no neurological

Table 1. Patients studied.

Neurological disorders	
SMON (No vitamin B_{12} therapy)	4
(Vitamin B_{12} treated)	19
Subarachnoid hemorrhage	4
Serous meningitis	3
Cerebral thrombosis	2
Polyneuritis	2
Spondylosis deformans	2
Cerebral hemorrhage	2
Epilepsy	1
Amyotrophic lateral sclerosis	1
Parkinsonism	1
Meniere's syndrome	1
Spinal tumor	1
Brain tumor	1
Controls	
No vitamin B_{12} treatment	27
Vitamin B_{12} treated	7
Megaloblastic anemia	
Vitamin B_{12} deficiency	1
Folic acid deficiency	3

diseases. The CSF was taken when spinal puncture was performed to exclude any cerebral and spinal disorder. Three patients with folic acid deficiency and 1 patient with pernicious anemia were also studied. Effects of a single injection of B_{12} on CSF levels of the vitamin were studied in two patients. Change of serum B_{12} levels after intrathecal injection of B_{12} was examined in a typical case of a SMON patient who had not received B_{12} previously.

Figure 1 shows the CSF B₁₂ levels in patients with various neurological diseases and the controls. No disease specific feature was seen in CSF B₁₂ levels. High

CSF B₁₂ levels were observed only in patients treated with B₁₂ for a long time. Twenty-seven controls that did not receive B₁₂ showed values ranging from 0 to 60 pg/ml (mean: 21.5). Effects of long term administration of B₁₂ on serum and CSF B₁₂ levels are shown in Table 2. Serum B₁₂ levels ranged from 875 to 20,000 pg/ml and CSF B₁₂ from 30 to 5,200 respectively. Except for two cases, CSF B₁₂ levels were below 300 pg/ml even when the serum B₁₂ was above 10,000 pg/ml. In patients treated with B₁₂, no correlation existed between serum and CSF B₁₂ levels. Normal levels of B₁₂ in CSF were only seen among patients treated with B₁₂ for less than three months. In order to see whether the transport of B₁₂ from serum to CSF occurs easily or not, CSF B₁₂ were assayed in two patients after a

Table 2. Effects of long term treatment with vitamin B₁₂ on the serum and cerebrospinal fluid vitamin B₁₂ levels.

Patients	Disease	Route of administration	Duration	Serum vit. B ₁₂ (pg/ml)	CSF vit. B ₁₂ (pg/ml)
1	SMON	INJ	over 6 M	3,000	88.7
2	"	"	3 M	12,000	50
3	"	"	1 M	12,000	30
4	"	"	over 3 Y	10,000	5,200
5	"	PO	6 M	1,450	43
6	"	INJ	1 Y	4,500	144
7	"	PO	2 Y	1,800	195
8	"	INJ	1.5 Y	20,000	240
9	"	"	8 M	3,100	170
10	"	"	3 Y	2,150	930
11	"	"	2 Y	2,200	266
12	Polyneuritis	"	1 M	12,000	50
13	Sjögren syndrome	"	6 M	1,500	85
14	Folic acid deficiency	"	1 M	2,375	60.7
15	"	PO	3 M	875	120

SMON, subacute myelo-optico-neuropathy; INJ, injection; PO, oral administration; M, month; Y, year; CSF, cerebrospinal fluid.

single injection of B₁₂. As shown in Table 3, no increase of CSF B₁₂ levels was observed in either patients 1 and 3 days after the injection of B₁₂, although serum B₁₂ levels were markedly increased. Reciprocally, intrathecal administration of B₁₂ also caused only slight elevation of serum B₁₂ as shown in Table 4. A delay of more than four hours was seen before serum B₁₂ level was elevated.

Table 3. Effect of single injection of vitamin B₁₂ on cerebrospinal fluid vitamin B₁₂ level.

Patient	Disease	Type of vit. B ₁₂ (amount)	Day of examination after injection	Serum vit. B ₁₂ (pg/ml)	CSF vit. B ₁₂ (pg/ml)
16	SMON	Hydroxocobalamin (250 µg)	1 day	10,000	17
17	Neurosis	Methylcobalamin (500 µg)	3 days	2,000	13.4

Table 4. Effect of intrathecal vitamin B₁₂ on the serum vitamin B₁₂ levels.

Serum vit. B ₁₂ before vit. B ₁₂ injection	Serum vit. B ₁₂ after					
	2 hr	4 hr	6 hr	12 hr	24 hr	48 hr
530	550	550	1,450	1,300	810	850

500 µg of deoxyadenosyl cobalamin was injected intrathecally in a patient with subacute myelo-optico-neuropathy.

DISCUSSION

B₁₂ levels in the CSF is only 5–10% of the level found in serum (1). Mean CSF B₁₂ levels on 27 control subjects was 21.2 pg/ml, well in agreement with previous reports (8, 11, 12, 19). Although significant correlation was demonstrated between serum and CSF B₁₂ levels in the reports of WORM-PETERSEN (8) and SIMPSON (11) and BASIL *et al.* (12), we avoided observing the correlation between serum and CSF B₁₂ levels in the present study because the microbiological assay is thought to be inadequate to have a good reproducibility at such a low concentration of B₁₂. The binding capacity of CSF protein has been examined by MEYER *et al.* (18) and WORM-PETERSEN (19). According to them, the B₁₂ binding capacity of CSF protein is higher than that of serum (18) and the nature of the protein is different from that in serum (19). As a matter of fact, many reports claimed that CSF B₁₂ levels were not significantly correlated with CSF protein levels, the present paper does not give any information on the relationship between CSF B₁₂ and CSF protein. Further studies on CSF B₁₂ and CSF protein are required.

No correlation between CSF B₁₂ levels and neurological disorders was demonstrated. Changes of CSF B₁₂ levels have been recorded in only two neurological diseases in literature. One is multiple sclerosis (elevation of CSF B₁₂) reported by SOBOTKA *et al.* (7), though not confirmed by others (8, 11, 12), and the other, pernicious anemia (low CSF B₁₂) (8, 9, 13, 14). In the present study, the CSF B₁₂ level of a patient with pernicious anemia was 13 pg/ml. This level is lower than the mean of the controls. Interestingly, the CSF B₁₂ levels of patients with SMON were not significantly low. This finding confirms our previous conclusion that B₁₂ deficiency was not seen in patients with SMON (5). Although UKYO (6) claimed that some disturbances were seen in the metabolism of B₁₂ in patients with SMON, it is rather difficult to assume such abnormalities by normal serum and CSF B₁₂ levels in the patients with SMON.

The elevation of CSF B₁₂ was demonstrated only in those patients receiving large amounts of B₁₂ for a long time (Table 2). There is very little information concerning the transport of B₁₂ from serum to CSF or *vice versa*. BASIL *et al.* (12) reported high CSF B₁₂ (60–120 pg/ml) in B₁₂-treated multiple sclerosis.

As shown in Table 3, a single injection of B₁₂ failed to elevate CSF B₁₂ levels 1 and 3 days after the injection. But in rabbits, WORM-PETERSEN (15) observed rapid elevation of CSF B₁₂ (4 times) after the injection of B₁₂ into the ear vein. Further observation of CSF B₁₂ levels shortly after the injection of B₁₂ is required to explain the discrepancy between the present result in man and that in rabbit. Intrathecal administration of B₁₂ elevated serum B₁₂ levels but to very slight degrees and only after four hours. This result coincides with the report of UKYO (6) using ⁵⁷CO-B₁₂. The delay of the elevation of serum B₁₂ after the intrathecal injection of B₁₂ suggests the presence of blood brain barrier for this vitamin confirming the report of BAUER *et al.* (16). Although the number of observations are small both in the present study and others (6, 15), the existence of a selective control

mechanism of the passage of B₁₂ across the blood brain barrier is strongly suggested.

REFERENCES

- 1) CHANARIN, I. (1969): The megaloblastic anaemias. Blackwell, Oxford, pp. 222.
- 2) IGATA, A., and TOYOKURA, Y. (1971): Subacute Myelo-Optiko-Neuropathie (SMON) in Japan. Zur Frage der Chinoform-Vergiftung. *Munch. Med. Wschr.*, **113**, 1062-1066.
- 3) NAKAE, K., YAMAMOTO, S., and IGATA, A. (1971): SMON in Japan. *Lancet*, **ii**, 510-513.
- 4) UKYO, S., WAKISAKA, G., and HASAMA, A. (1971): Subacute combined degeneration of the cord (SCDC) and ataxia in pernicious anemia. *Nihonrinsho (Jpn. J. Clin. Med.)*, **29**, 2284-2296.
- 5) HIRAKI, K., IWASAKI, I., UCHIDA, Y., MIYOSHI, I., NANBA, M., SEZAKI, T., and TAGUCHI, H. (1969): Epidemiological and clinical observations on the so-called encephalomyelitis with abdominal symptom (SMON) in Okayama district. *Naika (Intern. Med.)*, **24**, 1139-1146.
- 6) UKYO, S. (1971): Therapy of SMON by vitamin B₁₂—Course and prognosis. In The report of the council for research and investigation of SMON. No. 6, pp. 25-33.
- 7) SOBOTKA, H., CHRISTOFF, N., and BAKER, H. (1958): Vitamin levels in normal cerebrospinal fluid. *Proc. Soc. Expl. Biol. Med.*, **103**, 801-802.
- 8) WORM-PETERSEN, J. (1962): Vitamin B₁₂ in the serum and cerebrospinal fluid in neurological diseases. *Acta Neurol. Scand.*, **38**, 241-255.
- 9) GJERTSEN, J., and SCHRUMPF, A. (1962): B₁₂-vitamin i spinal vaesken ved forskjellige sykdommer. *Nord. Med.*, **67**, 162-163.
- 10) KIDD, H. M., GOULD, C. E. G., and THOMAS, J. W. (1963): Free and total vitamin B₁₂ in cerebrospinal fluid. *Can. Med. Ass. J.*, **88**, 876-881.
- 11) SIMPSON, C. A. (1964): Vitamin B₁₂ levels in the serum and cerebrospinal fluid in multiple sclerosis. *J. Neurol. Neurosurg. Psychiat.*, **27**, 174-177.
- 12) BASIL, W., BROWN, J. K., and MATTHEWS, D. M. (1965): Observations on vitamin B₁₂ in serum and cerebrospinal fluid. *J. Clin. Path.*, **18**, 317-321.
- 13) FRENKEL, E. P., MCCALL, M. S., and WHITE, J. D. (1971): An isotopic measurement of vitamin B₁₂ in cerebrospinal fluid. *Am. J. Clin. Path.*, **55**, 58-64.
- 14) FRENKEL, E. P., MCCALL, M. S., and SHEEHAN, R. G. (1973): Cerebrospinal fluid folate, and vitamin B₁₂ in anticonvulsant-induced megaloblastosis. *J. Lab. Clin. Med.*, **81**, 105-115.
- 15) WORM-PETERSEN, J., and POULSEN, E. (1961): Transport of vitamin B₁₂ from blood to cerebrospinal fluid. *Biochem. Pharmacol.*, **8**, 323-324.
- 16) BAUER, H., and HEINRICH, H. C. (1961): Vitamin B₁₂ metabolism in neurological disease. in Chemical pathways of the nervous system., ed. by Folch -Pi, J., Pergamon Press, Oxford, pp. 120-125.
- 17) Japan Vitamin Society (1960): A method for the assay of blood vitamin B₁₂. *Vitamins (Kyoto)*, **19**, 438-445.
- 18) MEYER, L. M., BRETCHER, R. W., and MULZAC, C. (1959): Co⁶⁰ vitamin B₁₂ binding capacity of normal human cerebrospinal fluid. *Proc. Soc. Expl. Biol. Med.*, **100**, 607-608.
- 19) WORM-PETERSEN, J. (1966): Cerebrospinal fluid binding capacity for cyano- and hydroxocobalamin. *Proc. Soc. Expl. Biol. Med.*, **121**, 24-27.