

Safety and Efficacy of Intravenous Ultra-high Dose Methylcobalamin Treatment for Peripheral Neuropathy: A Phase I/II Open Label Clinical Trial

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Abstract

Objective No clinically effective treatment for promoting peripheral axonal regeneration has yet been established. Several experimental studies *in vitro* and *in vivo* have shown that a high dose of methylcobalamin (MeCbl), an analogue of vitamin B12, promotes axonal growth in peripheral nerve injury. We herein assessed the safety and efficacy of an ultra-high dose MeCbl treatment for patients with peripheral neuropathy and chronic axonal degeneration.

Methods Fourteen patients with immune-mediated or hereditary neuropathy in the chronic progressive or stable phase were enrolled. MeCbl, 25 mg/day for 10 days followed by monthly 25 mg for 5 months, was intravenously administered. The patients were evaluated before and 1 year following treatment. The primary endpoints were safety and improvement in the Medical Research Council (MRC) sum score in at least two muscles of the 20 muscles. This trial is registered with the University Hospital Medical Information Network (UMIN) Center in Japan under the ID: UMIN000009359.

Results There were **no adverse effects** in twelve of the patients, whereas treatment was discontinued in two patients who had seborrheic dermatitis at 3 months and respiratory tract infection at 2 months, respectively. Therefore, twelve patients were evaluated for the primary outcomes; the MRC sum score was improved in seven of the patients and unchanged or worsened in the remaining five patients.

Conclusion **Intravenous ultra-high dose MeCbl treatment is a safe and potentially efficacious therapy for patients with peripheral neuropathy and chronic axonal degeneration.**

Key words: methylcobalamin, axonal regeneration, peripheral neuropathy

(Intern Med 53: 1927-1931, 2014)

(DOI: 10.2169/internalmedicine.53.1951)

Introduction

Currently, there is no clinically available agent for the promotion of peripheral axonal regeneration in patients with neuropathy. Experimental studies have shown that adult mammalian peripheral nerves can regenerate (1), but this dogma was based on rodent models. In rodent models, peripheral nerve regeneration was found to occur easily, pre-

sumably because the duration and distance of denervation are short. However, studies on peripheral neuropathy patients revealed difficulty in peripheral nerve regeneration. In patients with Guillain-Barré syndrome (GBS), 20% remain unable to walk independently 6 months after the disease onset (2). Additionally, in chronic inflammatory demyelinating polyneuropathy (CIDP), 13% of the patients have severe disability even after receiving treatment, largely due to secondary axonal degeneration (3).

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Received for publication October 20, 2013; Accepted for publication April 14, 2014

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We typically do not see adequate nerve regeneration following the standard dosing of methylcobalamin (MeCbl). However, the experimental efficacy of an ultra-high dose MeCbl treatment *in vitro* and *in vivo* has been reported (4-7). Recently, Okada et al. showed that a high dose of MeCbl promotes neurite outgrowth and neuronal survival, and its continuous administration enhances nerve regeneration in a rat sciatic nerve injury model (5). Similarly, Watanabe et al. reported that an ultra-high dose MeCbl therapy promotes nerve regeneration in an experimental model of acrylamide neuropathy (4).

However, the safety and efficacy of an ultra-high dose MeCbl treatment for humans has not yet been proven (8). Only one small clinical trial of an ultra-high dose MeCbl treatment for amyotrophic lateral sclerosis (ALS) patients was reported (9). We herein report a prospective study designed to assess the safety and efficacy of ultra-high dose of MeCbl for the treatment of neuropathies with axonal degeneration.

Materials and Methods

Patients

A total of 14 patients with peripheral neuropathy were enrolled in this study. All patients gave informed consent for the protocol approved by the Institutional Review Board at Chiba University School of Medicine. This trial was registered with the University Hospital Medical Information Network (UMIN) Center in Japan under the ID: UMIN 000009359.

Patients with peripheral neuropathy, long disease duration (>3 years) and muscle atrophy, and electrodiagnostic evidence for axonal degeneration were included. Before the trial, a stable condition was confirmed in all patients for at least 3 months. Of the 14 patients, 3 had CIDP, 2 GBS [1 acute inflammatory demyelinating polyneuropathy (AIDP) and 1 acute motor axonal neuropathy (AMAN)], 8 Charcot-Marie-Tooth disease (CMT) and 1 radiation neuropathy. CIDP patients were resistant to therapy with intravenous immunoglobulin, plasmapheresis, prednisolone or immunosuppressant drugs and had a chronic progressive course. We included patients with various diseases because this study was a preliminary phase I/II open study.

Treatment protocol

Patients received the intravenous (i.v.) administration of MeCbl 25 mg/day for 5 days a week during the first 2 weeks. After 2 weeks, MeCbl 25 mg/day i.v. was administered monthly for the next 5 months.

Assessments

A clinical and laboratory evaluation was performed before treatment and at 12 months following treatment. The primary endpoints were the safety and Medical Research Council (MRC) sum score in 20 muscles. The secondary

endpoints included the overall neuropathy limitation scale (ONLS), grip strength and compound muscle action potential (CMAP) amplitudes. Patients who showed an increased MRC score in at least two muscles, improved ONLS, >50% increase in grip strength or increased sum CMAP amplitudes in 4 nerves (median, ulnar, tibial and peroneal nerve) were judged as responders (10-13).

The muscle strength was measured in 10 muscle groups (proximal: abduction of the arm, extension of the forearm, flexion of the forearm, flexion of the leg, extension of the knee and flexion of the knee; distal: extension of the wrist, flexion of the wrist dorsal flexion of the foot and plantar flexion of the foot) on both sides according to the MRC scale (maximum score: 100). The ONLS is a scale that measures limitations in the everyday activities of the upper and lower limbs (14). In the ONLS, a score of 0 indicates no limitations (the ceiling of the scale) and a score of 12 indicates no purposeful movement in the upper and lower limbs. The CMAPs were recorded from the abductor pollicis brevis, abductor digiti minimi, extensor digitorum brevis and abductor hallucis muscles after distal stimulation at the wrist or ankle. The amplitudes were measured from the baseline to the negative peak. The data on muscle strength and the ONLS were measured by one of the authors (K.S.) who was not blinded to the clinical information. The sensory examination was not evaluated in this study due to the difficulty in quantifying sensory loss.

Serum vitamin B12 assay

The serum vitamin B12 concentrations were measured before and 10 days after the initiation of treatment using a chemiluminescence enzyme immunoassay (CLEIA, normal range: 180-914 pg/mL). On the 10th day, a serum sample was taken before MeCbl injection. In patient no. 11, information on the serum vitamin B12 concentrations was reported as "over 1,500 pg/mL" following treatment.

Statistical analysis

All statistical analyses were performed using the SAS V.4.2 software program. The data were compared before and 12-months following treatment using the paired Student's *t*-test. The level of statistical significance was set at $p < 0.05$.

Results

Two patients withdrew from the trial due to adverse events. One CIDP patient withdrew due to pneumonia at four months from the start of the trial, whereas one CMT patient dropped out due to seborrheic dermatitis at two months from the start. The CIDP patient had a disease history for longer than 20 years, severe atrophy and respiratory muscle involvement; therefore, the pneumonia was speculated to be related to the CIDP. Moreover, the dermatitis was not judged to be related to the MeCbl treatment by a dermatologist.

Table. Results of Endpoint

| Patient | Diagnosis | Age/ gender | Duration (years) | MRC score | | ONLS | | Grip sum (kg) | | CMAP amplitude sum (mV) | | | |
|------------|----------------------|----------------|---------------------|-----------|--------|-------------|--------|---------------|-------------|-------------------------|--------|-------------|------|
| | | | | Before | 1 year | Improvement | Before | 1 year | Improvement | Before | 1 year | Improvement | |
| 1 | AIDP | 61F | 3 | 78 | 90 | 5 | 4 | 0 | 2 | 2 | 6.8 | 9.8 | 3.8 |
| 2 | CIDP | 56M | 20 | 83 | 85 | 8 | 8 | 15 | 7 | -8 | 0.9 | 0.4 | -0.5 |
| 3 | CIDP | 27M | 17 | 76 | 78 | 4 | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | CMT1A | 44M | 14 | 90 | 92 | 4 | 4 | 53 | 49 | -4 | 2.3 | 5.3 | 3 |
| 5 | CMT2 | 65M | 10 | 82 | 84 | 4 | 4 | 46 | 53 | 7 | 14.6 | 14.2 | -0.4 |
| 6 | CMT3 | 24F | 24 | 86 | 88 | 3 | 3 | 34 | 38 | 4 | 0.5 | 1.7 | 1.2 |
| 7 | CMT3 | 31F | 29 | 68 | 70 | 6 | 6 | 6 | 6 | 0 | 2.4 | 1.6 | -0.8 |
| 8 | CMT1A | 44M | 22 | 92 | 93 | 6 | 6 | 50 | 50 | 0 | 6 | 3.5 | -2.5 |
| 9 | CMT1A | 25M | 9 | 100 | 100 | 2 | 2 | 41 | 62 | 21 | 8.2 | 9.3 | 1.1 |
| 10 | AMAN | 61M | 50 | 90 | 90 | 5 | 5 | 34 | 52 | 18 | 10.5 | 7.3 | -3.2 |
| 11 | Radiation neuropathy | 59M | 24 | 88 | 86 | 3 | 3 | 96 | 93 | -3 | 17.2 | 15.7 | -1.5 |
| 12 | CMT3 | 42F | 24 | 48 | 44 | 10 | 11 | 0 | 0 | 0 | 0 | 0 | 0 |
| Mean value | | 45 | 21 | 82 | 83 | 5 | 5 | 31 | 34 | 3.1 | 5.8 | 5.7 | |
| p value | | | | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |

CIDP: chronic inflammatory demyelinating polyneuropathy, AIDP: acute inflammatory demyelinating polyneuropathy, AMAN: acute motor axonal neuropathy, CMT: Charcot-Marie-Tooth, MRS: Medical research council, ONLS: over all neuropathy limitation scale, CMAP: compound muscle action potential

The primary endpoint

The results of the MRC sum score are indicated in Table. Seven patients (nos. 1-7) showed an increased MRC score in at least two muscles. All of the patients had moderate weakness at entry (MRC score: 68-90). Conversely, four patients (nos. 8-12) who had severe or mild weakness at the start of the trial did not show improvement. Most of the immune-mediated neuropathy patients showed an increased MRC score. One AIDP patient (no. 1), showed a 12-point improvement. The average MRC score 1 year following treatment was similar to the scores seen before the trial. The vitamin B12 levels prior to treatment ranged from 201 to 1,120 pg/mL, whereas those after therapy were markedly elevated, ranging from 887,000 to over 2 million pg/mL.

The secondary endpoint

Only one patient (no. 1) showed improvement in the ONLS (Table). This patient showed clear improvement in all of the endpoints. Before treatment, she could not walk more than 5 meters or fasten buttons for 3 years; however, following treatment, she acquired the ability to walk more than 10 meters and lace up her shoes. Three patients (nos. 1, 9 and 10) demonstrated an increased grip strength, and 3 patients (nos. 1, 4 and 6) showed increased CMAPs.

Discussion

Our results show that intravenous ultra-high dose methylcobalamin is a safe and potentially efficacious treatment for patients with peripheral neuropathy. During the study period of 12 months, administration of an ultra-high dose MeCbl did not result in serious adverse events. Improvement in the clinical and laboratory measures was mild, but the results raise the possibility of the effectiveness of ultra-high dose MeCbl in some patients.

Our trial was safely performed. Two patients experienced adverse events, however, this was most likely not related to the MeCbl treatment. A previous clinical trial of ultra-high dose MeCbl therapy for ALS patients showed that, of 12 patients, one patient had a skin rash and one had mild elevation of the serum liver enzyme levels (9). In our trial, such side effects were not observed. Another clinical trial for chronic hemodialysis patients with uremic or diabetic neuropathy revealed the safety of vitamin B12 (15): the typical dose of MeCbl was administered for chronic hemodialysis patients, and the serum concentrations of vitamin B12 were ultra-high due to the lack of urinary excretion in these patients. To establish the safety of an ultra-high dose MeCbl therapy, a larger prospective study is necessary; however, both our present results and those in the pertinent literature suggest the safety of an ultra-high dose MeCbl treatment.

Our trial suggests the effectiveness of an ultra-high dose MeCbl treatment for a subgroup of patients. Patients with moderate weakness tended to improve, and immune-mediated neuropathy patients also tended to show clinical

improvement. Specifically, one AIDP patient (no.1) dramatically improved after the trial, although she had severe disabilities for the past 3 years. This case may suggest a potential power of the ultra-high dose MeCbl therapy. Conversely, there was no obvious improvement in patients with CMT, and this may be because the treatment effects did not overcome a degenerative progressive condition in the disorder.

The mechanisms of nerve repair with MeCbl treatment are still not clearly understood, but various hypotheses have been postulated in several reports. First, MeCbl increases the Erk1/2 and Akt activities through the methylation cycle and promotes nerve regeneration (5). Recently, Okada et al. showed that MeCbl and S-adenosylmethionine (SAM) have similar effects on neurite outgrowth, SAM did not increase the effect of MeCbl, and 4-nitro-2, 1, 3-benzothiadiazole only inhibited the effects of MeCbl. These results suggested that MeCbl promotes nerve regeneration through increasing the expression of Erk1/2 and Akt. In this study, only the high serum concentration of vitamin B12 promoted neurite outgrowth and neuronal survival. Second, MeCbl acts on Schwann cells to promote axonal regeneration (6, 16). Some reports suggested the importance of the Schwann tube (Schwann cell basal lamina) (17-19); the Schwann cell basal lamina is rich in extracellular matrix molecules that promotes axonal growth and serves as a reservoir of growth factors secreted by the Schwann cells. Third, MeCbl promotes RNA and protein synthesis (6). These effects work on the motoneurons to activate protein synthesis. In conclusion, all or some of these mechanisms could cause the nerve terminal to regenerate.

In our study, all patients had extremely high vitamin B12 concentrations following MeCbl injection. Okada et al. revealed that MeCbl increases the axonal length and decreases the apoptotic rate in cerebellar granule neurons only at concentration >100 nM (approximately 130, 000 pg/mL) (5). Almost all of our patients achieved this concentration. The efficacy may depend on the extent of the elevation of the vitamin B12 concentration.

Our study has some major limitations. We conducted an open, non-blinded trial that enrolled a small number of patients. Moreover, we cannot exclude the possibility of placebo effects and natural recovery in some of the patients. Clinical trials enrolling a larger number of patients are necessary to completely determine the safety and efficacy of an ultra-high dose MeCbl treatment. Moreover, the sensitivity and reproducibility of the CMAP amplitudes and grip strength are not sufficiently high, and more sensitive and reliable parameters are required in future studies.

For CMT disease, ascorbic acid, progesterone and HDAC6 inhibitors have been shown to be beneficial in animal models (20-23) but not in clinical trials (21, 24-26). Such specific treatments according to the pathophysiology of each neuropathy are necessary. However, symptomatic treatments (even non-specific) are absolutely necessary for patients with peripheral neuropathy who still have a disability due to axonal degeneration. We therefore recommend an

ultra-high dose of MeCbl as a viable a treatment option and suggest that a large randomized controlled trial be conducted for each neuropathy in the near future.

The authors state that they have no Conflict of Interest (COI).

Acknowledgement

Dr. Misawa received research support from the Ministry of Education, Culture, Sports, Science, and Technology of Japan. Dr. Kuwabara received research support from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, and Grants-in-Aid from the Research Committee of CNS Degenerative Diseases, and the Research Grant 16B-1 for Nervous and Mental Disorders from the Japanese Ministry of Health, Labour and Welfare.

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