Journal of the Neurological Sciences 354 (2015) 70–74

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns

Neuroprotective effect of ultra-high dose methylcobalamin in wobbler mouse model of amyotrophic lateral sclerosis

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ARTICLE INFO

Article history:
Received 2 February 2015
Received in revised form 8 April 2015
Accepted 28 April 2015
Available online 8 May 2015

Keywords:
Methylcobalamin
Ultra-high doses
Wobbler mouse
Amyotrophic lateral sclerosis
Motor neuron disease
Homocysteine animal model

ABSTRACT

Background: High-dose of methylcobalamin promotes nerve regeneration in rats with acrylamide neuropathy. A double-blind controlled trial suggested that high-dose methylcobalamin could increase compound muscle action potentials in patients with amyotrophic lateral sclerosis (ALS). A large-scale extended period human trial is now on-going in ALS (Clinicaltrial.gov NCT00444613). We attempted to study whether high-dose methylcobalamin can improve symptoms or retard progression of motor dysfunction in the wobbler mouse model of ALS.
Methods: After initial diagnosis of the disease at the postnatal age of 3–4 weeks, wobbler mice received methylcobalamin (3 or 30 mg/kg, n = 10/group) or vehicle (n = 10), daily for 4 weeks by intraperitoneal administration in a blinded fashion. We compared clinical symptoms and pathological changes among all groups. Vitamin B12 concentrations were measured in the serum, the skeletal muscle and the spinal cord of three groups (n = 5/group).
Results: In comparison with vehicle, mice treated with ultra-high dose (30 mg/kg) of methylcobalamin significantly inhibited muscle weakness and contracture in the forelimb, and increased the weight of the bicep muscles and the number of musculocutaneous nerves. Methylcobalamin-treated mice significantly elevated vitamin B12 concentrations of the serum, the bicep muscle and the spinal cord compared to vehicle.
Conclusion: Our results suggest that treatment with methylcobalamin could delay progression of motor symptoms and neuropathological changes in wobbler mouse motor neuron disease if very high doses are used.

1. Introduction

Usual dose of methylcobalamin (0.5 mg i.v.) has been applied for supplementing vitamin B12 deficiency, especially for those who had gastrectomy to prevent anemia and subacute combined degeneration of the spinal cord. On the other hand, ultra-high dose (25 mg/day i.v.) has been shown to improve muscle weakness in patients with peripheral neuropathy [1]. Methylcobalamin promotes nerve regeneration in rat peripheral neuropathy or injury models [2–5]. A previous clinical trial demonstrated short-term high-dose (25 mg/day i.m.) methylcobalamin increased the compound muscle action potential amplitudes in patients with amyotrophic lateral sclerosis (ALS) [6]. A large-scale extended period trial is now on-going in ALS patients (Clinicaltrial.gov NCT00444613).

Wobbler mouse revealed neuromuscular deficits and neuropathological findings partially mimicking ALS [7–10]. We previously examined therapeutic efficacies of several agents in wobbler mice [11–15]. The present study was aimed to evaluate clinical and morphometric changes of wobbler mice treated with high dose (3 mg/kg/day) and ultra-high dose (30 mg/kg/day) of methylcobalamin. We also examined the serum, the skeletal muscle and the spinal cord concentration of vitamin B12 in methylcobalamin- and vehicle-treated wobbler mice.

2. Methods

2.1. Animals

The original wobbler mouse mutation (wr) occurred spontaneously as an autosomal recessive mutation in the congenic C57BL/6J mouse strain [7]. The original colony was crossed with a high fertility strain (NFR/N) and maintained. Wobbler mice (wr/wr) and their normal littermates (wr/+ or +/+) in this study were raised in the Fourth Department of Internal Medicine, Toho University mouse colony, Tokyo, Japan. This colony was donated by Prof. Hiroshi Mitsumoto from the Department of Neurology, Columbia University, USA. The protocol was approved by the Institutional Animal Care and Use Committee at Toho University (#2000-126).

2.2. Methylcobalamin administration

Methylcobalamin (Sigma) was prepared with special caution to keep in a dark place, because of its light-sensitivity. The wobbler mice initially developed body trembling from the age of 3 to 4 weeks, and

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http://dx.doi.org/10.1016/j.jns.2015.04.052
0022-510X/

were diagnosed as the onset of disease. Immediately after diagnosis, affected mice received two doses of methylcobalamin (3 mg/kg, n = 10; 30 mg/kg, n = 10) or vehicle (n = 10) by intraperitoneal administration daily for 4 weeks in a blinded fashion. The treatment finished at age 7 to 8 weeks.

2.3. Assessment of clinical symptoms

Forelimb deformity was graded as 1, paw atrophy; 2, curled digits; 3, curled wrists; and 4, forelimb flexion to chest. Forelimb strength of forelimbs was measured as described previously [11–15]. These assessments were performed weekly from the beginning (pre-treatment) to the end of treatment in a blinded fashion.

2.4. Morphometry of bicep muscle and musculocutaneous nerve

After treatment, wobbler mice (n = 10/group) were anesthetized with ether and intraperitoneal injection of pentobarbital sodium (40 mg/kg), and the right bicep muscles were removed under a dissecting microscope. They were accurately weighed and frozen. Serial 10 μm sections were stained with NADH. The mean diameter of muscle fibers was determined. Morphometric analysis was also done in five age-matched healthy littermates for comparison.

After dissection of the right bicep muscles, the left musculocutaneous nerve at the bicep muscle insertion (the motor nerve for the bicep muscle) was sampled under a dissecting microscope. Transverse sections, 0.5 μm thick, were prepared from the musculocutaneous nerves. The total number and size of myelinated fibers in the musculocutaneous nerve were determined in three experimental groups and age-matched normal mice (n = 5), using morphometric system, MacScope®.

2.5. Number of spinal motor neurons

Wobbler mice (n = 10/group) that were used for morphometric analysis of bicep muscles and musculocutaneous nerves were perfused through an intracardiac catheter with phosphate buffered saline followed by 4% paraformaldehyde/1% glutaraldehyde/0.1 M sodium phosphate buffer, pH 7.4. Laminectomy was performed, and the cervical spinal cord was removed under a dissecting microscope. C5–6 segments, which innervate the bicep muscles, were taken for analysis of motor neurons. The spinal cord segments were embedded in paraffin, sectioned serially at 8 μm in the transverse plain, and stained with cresyl-violet. The number of motor neurons was determined as described [11–15].

2.6. Concentrations of vitamin B12 in the serum, the skeletal muscle and the spinal cord

Intraperitoneal administration of methylcobalamin (3 or 30 mg/kg, n = 5/group) or vehicle (n = 5) was performed in wobbler mice daily for 4 weeks. At 1 h following the final administration, blood was obtained from the abdominal vein under ether anesthesia and intraperitoneal injection of pentobarbital sodium (40 mg/kg). The bicep muscle and the spinal cord were excised. The tissue was washed with saline, weighed and homogenized. The vitamin B12 concentrations were determined by microbiological assay using *Lactobacillus delbruekii* subsp. lactis ATCC 7870. The analysis was also done in five age-matched normal mice without methylcobalamin treatment.

2.7. Statistical analyses

All data are shown as mean ± SEM. The differences between vehicle group and methylcobalamin groups in the scale of forelimb deformity and grip strength were analyzed by the repeated measures ANOVA followed by Dunnett multiple comparison test. The bicep muscle weight, the number of musculocutaneous nerves and motor neurons, and the concentrations of vitamin B12 were analyzed using one-way ANOVA followed by Dunnett multiple comparison tests. The differences between vehicle wobbler group and normal mice group in the concentration of vitamin B12 were analyzed by unpaired t test. A P value of <0.05 (two-sided) was considered to be significant. Statistical analyses were performed using Graph Pad Prism (Version 6.02).

3. Results

3.1. Clinical symptoms

The assessments at baseline (postnatal age 3–4 weeks) did not differ among the three groups. Forelimb deformity deteriorated progressively in vehicle-treated mice, whereas treatment with ultra-high dose of methylcobalamin delayed the progression of deformity (Fig. 1A). The mean ± SEM of forelimb deformity at the 2nd and 4th weeks was 1.6 ± 0.2 grade and 2.9 ± 0.2 in the vehicle group, 1.5 ± 0.2 and 2.6 ± 0.3 in the high dose group, and 1.2 ± 0.2 and 2.1 ± 0.3 in the ultra-high dose group, respectively.

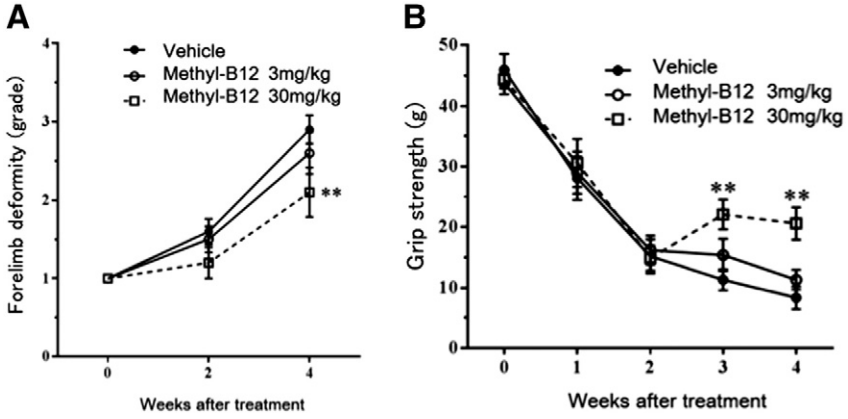

Fig. 1. (A) Symptomatic effects of methylcobalamin on forelimb deformity. Forelimb deformity in all groups is scored as grade 1 at pretreatment. Ultra-high doses of methylcobalamin retarded progression of the deformity. $^{**}P < 0.01$ versus vehicle group (repeated measures ANOVA followed by Dunnett type multiple comparison test in the 4th week). Data in the 1st and 3rd weeks are not shown. Results are represented as the mean ± SEM (n = 10/group). (B) Symptomatic effects of methylcobalamin on forelimb strength. Administration of ultra-high dose methylcobalamin delayed deterioration of grip strength. $^{**}P < 0.01$ versus vehicle group (repeated measures ANOVA followed by Dunnett type multiple comparison test in the 3rd and 4th weeks). Results are represented as the mean ± SEM (n = 10/group).