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Effectiveness of Metanx Prescription Medical Food on Small Nerve Fibers and Monofilament Sensation in Patients with Diabetic Peripheral Polyneuropathy

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Abstract

Purpose: Diabetic peripheral neuropathy (DPN) is prevalent among the population with type 2 diabetes, and treatment approaches are limited. The combination of L-methylfolate-methylcobalamin-pyridoxal-5-phospate (LMF-MC-PP, Metanx®) is a prescription medical food that has demonstrated significant improvements in sensory perception and quality of life as well as reduced neuropathic pain in patients with DPN. The present study examined the effects of LMF-MC-PP on sensory perception and epidermal nerve fiber density (ENFD) among patients with confirmed DPN. Methods: Patients with type 2 diabetes and diagnosed with diabetic peripheral polyneuropathy, based on loss of vibratory perception, warm-cold discrimination or monofilament sensation, underwent bilateral lower extremity ENFD assessments via skin punch biopsy and were started on LMF-MC-PP. ENFD and monofilament testing were repeated at 6 months. Findings: Of 123 patients evaluated, all had monofilament testing at baseline and 6 months and 122 had assessments at both time points. A significant ($p < 0.05$) improvement in ENFD from baseline to 6 months was observed. A significant ($p = 0.0001$) improvement in monofilament testing also was observed. Overall, more patients had intact sensation after 6 months, with 60 (48.8%) of 123 patients having positive monofilament test at baseline as compared to 95 (77.2%) positive after 6 months. An analysis of the association between improvements in ENFD and monofilament testing found that the two tended to occur together, and this was significant ($p < 0.05$) for the right limb. Implications: Clinically important and statistically significant improvements in ENFD and monofilament sensation were associated with LMF-MC-PP in patients with DPN. When compared to the decrease in ENFD expected among DPN patients, the improvements are even more clinically sig-

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nificant. These findings should be validated in a larger, placebo-controlled study.

Keywords

Diabetic Neuropathy, Epidermal Nerve Fiber Density, LMF-MC-PP, Monofilament Testing, Small Nerve Fibers

1. Introduction

Diabetic sensorimotor peripheral neuropathy (DSPN) is prevalent among the population of patients with type 2 diabetes occurring in up to 50% of diabetic patients [1]-[6]. Factors associated with DSPN include age, duration of diabetes and severity of hyperglycemia [1] [6]-[9]. No treatments modify the underlying disease; existing treatment options focus only on symptomatic relief of pain. Thus, a need exists for effective treatments that modify the underlying disease state as well as improve symptoms [10]-[12].

L-methylfolate calcium is the biologically active form of folic acid and may have a role in improving endothelial function and correcting the underlying pathology of DPN [13]. The rationale for using LMF combined with vitamins B12 and B6 as potential disease modifying therapy is based on results from preclinical and clinical studies showing improved endothelial function, reduced oxidative stress and improvements in objective measures in animal models of neuropathy [14]-[21].

The combination of L-methylfolate calcium-methylcobalamin-pyridoxal-5-phospate (LMF-MC-PP, Metanx, Nestlé Health Science - Pamlab, Inc., Covington, LA) is a prescription medical food. Previous studies with LMF-MC-PP in patients with DPN have reported significant improvements in neuropathic pain, quality of life, as well as sensory perception as measured by 1-point (tactile) and 2-point (sensory discrimination) tests [22]-[27]. The largest study by Fonseca *et al.* demonstrated that LMF-MC-PP significantly improved neuropathic symptoms and quality of life in DPN patients but did not affect vibration perception threshold compared to placebo [22]. In a case series of 11 DPN patients treated with LMF-MC-PP who underwent repeat skin punch biopsies, treatment for 6 months with LMF-MC-PP was associated with a significant improvement in epidermal nerve fiber density (ENFD) [27].

The objective of this study was to evaluate the effects of LMF-MC-PP on ENFD and monofilament sensation in patients with diabetic neuropathy. Monofilament sensation testing has been shown to predict ulceration and amputation in patients with DPN [28]-[34] and is therefore recommended by the American Diabetes Association (ADA) and American Association of Clinical Endocrinologists (AACE) as a part of the assessment and management of diabetic neuropathy [35]-[37].

2. Methods

2.1. Study Design

This was an observational study of patients presenting to a community podiatry clinic with type 2 diabetes and clinical DPN who were selected to undergo skin biopsies to evaluate ENFD before and after treatment with LMF-MC-PP. The study protocol was reviewed by an institutional review board that granted a waiver of informed consent. All patient information was de-identified prior to collection.

2.2. Patient Selection

Patients with type 2 diabetes and diagnosed with diabetic peripheral polyneuropathy based on vibratory sensorium, warm-cold discrimination or loss of protective sensorium, and an ENFD assessment were included. Patients with a medical history or laboratory tests that documented chemotherapy, heavy metal poisoning, pernicious anemia, sero-positive or sero-negative arthropathy or any systemic metabolic inflammatory disease process other than cardiovascular disease were excluded from this study.

2.3. Study Assessments

A screening qualitative neurological examination was conducted using all of three methods. Abnormal vibratory

sensorium—a 128 C tuning fork was held to the dorso-medial bony eminence of the #1 interphalangeal joint of each foot. When the patient reported that no vibration was any longer evident, the tuning fork was moved to the analogous articulation of the thumb and the response was noted. Abnormal warm-cold discrimination—separately, two different metals of known different heat capacities were applied in sequence to the plantar tuft of the Hallux. The patient was requested to identify which felt warmer and which felt colder. This technique was applied to the palm of the hand, in sequence, after the toe, to confirm the response of the difference between the two metals. Protective sensorium loss—an 11-point load test of 10 gm/cm^{2}was applied to the dorsal and plantar aspects of the feet and toes following the standard Semmes-Weinstein Monofilament (SWM) technique [37]. If any of these three qualitative neurological parameters were abnormal, the patient underwent an skin biopsy for ENFD quantitation.

ENFD analysis was conducted on each leg via a 3mm punch biopsy of skin, 10cm superior to the tip of the lateral malleolus, utilizing established protocols for atraumatic technique to quantify the extent of small nerve fiber loss. The patient was placed in a left lateral decubitus position. An impression in the skin was made in the lateral right ankle at a height of 10.5 cm superior to the lateral malleolus. Isopropyl alcohol was applied to the skin without rubbing, and topical ethyl chloride was applied to chill only the skin superior to the impression site prior to injection. The area superior to skin impression was atraumatically anesthetized with 2% lidocaine with epinephrine 1 cc taking care to avoid the biopsy site. When adequate anesthesia was established, a sterile 3 mm skin punch was gently used to dissect the biopsy from the surrounding skin. Utilizing atraumatic forceps and iris scissors and without touching the skin in the process, the biopsy was removed and placed in a marked fixative vial. Between 6 and 24 hours later, the biopsy was removed from the fixative, rinsed, and placed into dry ice for shipping to Bako Pathology (Alpharetta, GA) following standard protocol and technique. A steri-strip adhesive bandage and antibiotic dressing was applied to the wound. The patient was placed in the right lateral decubitus position, and the procedure was repeated for the left ankle biopsy. The patient was advised verbally and in writing of the correct wound care protocol for the biopsy sites.

Upon receipt of the bilateral ENFD analysis results from Bako Pathology (usually within 2 weeks), the results and their clinical significance were discussed with the patient. An abnormal ENFD analysis was considered one in which the SNF count was lower than 7.1 fibers/mm and/or morphological degenerative changes were noted. If an abnormal ENFD analysis of either lower extremity was obtained, a prescription for LMF-MC-PP (L-methylfolate calcium 3 mg; pyridoxal 5'-phosphate 35 mg, methylcobalamin 2 mg) medical food one capsule twice daily was prescribed, and the patient was encouraged to persist with therapy. The assessment was performed 6 months after the initial analysis to determine the effect of LMF-MC-PP on ENFD. The Semmes-Weinstein Monofilament test was also repeated at 6 months.

2.4. Statistical Analysis

Descriptive statistics were calculated including means, standard deviations for continuous variables, and frequencies and percentages for categorical variables.

Two outcome measures were studied: 1) small nerve fiber density and 2) monofilament test results for sensation. These measures were evaluated at baseline (*i.e.* before the initiation of LMF-MC-PP therapy) and at 6 months post-baseline for both the right and left legs. For ENFD, the change in the number of fibers per millimeter was calculated, and paired t-tests were carried out to evaluate the significance. For monofilament test results, the presence/absence of sensation was analyzed. The Kappa coefficient and Bowker's test were used to evaluate the significance of change. The correlation of change from baseline to 6 months between ENFD and monofilament test was investigated with a Spearman's correlation coefficient. A significant result was considered $p < 0.05$.

3. Results

A total of 123 diabetic patients were evaluated and treated between 2010 and 2014 and were included in the analysis. Monofilament testing results were available for all 123 patients and ENFD analysis results were available for 122. Baseline and 6-month data for ENFD results were available for 111 patients for the dominant limb analysis and 110 patients for the non-dominant limb assessment. Baseline demographic and clinical characteristics reveal a population that was consistent with patients having type 2 diabetes (**Table 1**). Two-thirds were at least 65 years of age, and all were treated with some type of hypoglycemic therapy.

Table 1. Baseline characteristics.

	N (%)
Baseline Year	123
2010	61 (49.6)
2011	25 (20.3)
2012	20 (16.3)
2013	15 (12.2)
2014	2 (1.6)
Age, years	
<65	45 (36.6)
≥65	78 (63.4)
Gender	
Female	49 (39.8)
Male	74 (60.2)
Ethnicity	
Non-hispanic	105 (86.1)
Hispanic or Latino	17 (13.9)
Diabetes Treatment	
Diet	9 (7.3)
Metformin	91 (74.0)
Insulin	31 (25.2)
Other	50 (40.7)

3.1. Epidermal Nerve Fiber Density Analysis

A significant ($p < 0.05$) improvement from baseline to 6 months was observed in the ENFD analysis for the right limb, left limb, dominant limb, and non-dominant limb (Table 2). The mean magnitude of change was at least 0.6 nerve fibers/mm for the right limb and dominant limb but reached 1.1 nerve fibers/mm for left limb.

3.2. Monofilament Sensation Testing

A significant ($p = 0.0001$) improvement in monofilament sensation testing was observed with LMF-MC-PP treatment. Of 63 patients with absent sensation at baseline, 38 (60.3%) had intact sensation after 6 months of treatment with LMF-MC-PP. In contrast, only three (5.0%) of 60 patients with intact sensation at baseline lost sensation after 6 months of treatment (Table 3). Overall, 60 (48.8%) of 123 patients had intact sensation at baseline, and 95 (77.2%) had intact sensation at 6 months (Figure 1). Not more than three patients (depending upon which limb) experienced worsening sensation at six months.

3.3. Correlation between ENFD and Monofilament Sensation Testing

An analysis was conducted of the association between monofilament sensation improvement and ENFD improvement where the latter was defined as an increase from baseline of at least 1.0 fibers/mm. Among those with ENFD improvement, 33.3% (left foot; $p = 0.44$) and 44.2% (right foot; $p = 0.02$) of patients also improved in sensation at six months (Table 4). When ENFD improvement was defined as 0.5 fibers/mm (Table 5), then the respective percentages were 34.7% (left foot; $p = 0.26$) and 41.7% (right foot; $p = 0.03$).

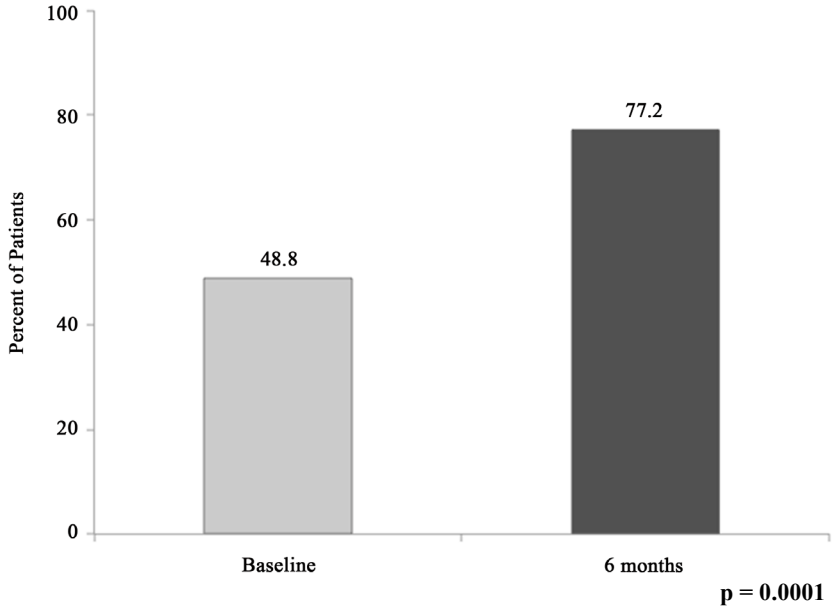

Figure 1. Patients with intact sensorium before and after LMF-MC-PP.

Table 2. Analysis of ENFD results at baseline, after 6 months, and change from baseline to 6 months.

	N	Mean ± SD (# fibers/mm)	p-value*
Right Foot Baseline	122	5.2 ± 5.2	
Right Foot 6 Month	122	5.7 ± 6.0	
Right Foot Change from Baseline	122	0.6 ± 3.7	0.0433
Left Foot Baseline	123	4.7 ± 4.4	
Left Foot 6 Month	122	5.7 ± 5.8	
Left Foot Change from Baseline	122	1.1 ± 3.4	0.0004
Dominant Limb Baseline	111	5.1 ± 5.1	
Dominant Limb 6 Month	111	5.7 ± 5.9	
Dominant Limb Change from Baseline	111	0.6 ± 3.8	0.0393
Non-dominant Limb Baseline	111	4.8 ± 4.4	
Non-dominant Limb 6 Month	110	5.9 ± 5.8	
Non-dominant Limb Change from Baseline	110	1.0 ± 3.4	0.0012

*One-sided test for improvement at 6-month follow-up.

Table 3. Monofilament test results.

Baseline	6 Month	Change at 6 month	N (%)	Agreement Statistic	Symmetry of Disagreement
				Kappa Coefficient	Bowker's Test
Negative	Negative	Negative to Negative	25 (20.3)	0.24, p = 0.0001	p = 0.0001
	Positive	Negative to Positive	38 (30.9)		
Positive	Negative	Positive to Negative	3 (2.4)		
	Positive	Positive to Positive	57 (46.3)		

Table 4. Correlation between SNF and monofilament testing. Proportion of patients with type 2 diabetes with improvement of at least 1.0 mm/fiber from baseline.

	10 g Changes					
	Improved	No Change	Worsened	Total	Measures of Association	
	N (%)	N (%)	N (%)	N	Spear-man's	p-value
	Right foot difference from baseline				0.219	0.0156
≥1 Improvement	19 (44.2)	24 (55.8)	0 (0.0)	43		
<1 Improvement	19 (24.1)	58 (73.4)	2 (2.5)	79		
Total	38 (31.2)	82 (67.2)	2 (1.6)	122		
	Left foot difference from baseline				0.071	0.4352
≥1 Improvement	14 (33.3)	28 (66.7)	0 (0.0)	42		
<1 Improvement	23 (28.8)	54 (67.5)	3 (3.8)	80		
Total	37 (30.3)	82 (67.2)	3 (2.5)	122		
	Dominant limb difference from baseline				0.149	0.1180
≥1 Improvement	17 (40.5)	25 (59.5)	0 (0.0)	42		
<1 Improvement	19 (27.5)	48 (69.6)	2 (2.9)	69		
Total	36 (32.4)	73 (65.8)	2 (1.8)	111		
	Non-dominant limb difference from baseline				0.107	0.2647
≥1 Improvement	14 (37.8)	23 (62.2)	0 (0.0)	37		
<1 Improvement	21 (28.8)	50 (68.5)	2 (2.7)	73		
Total	35 (31.8)	73 (66.4)	2 (1.8)	110		

Table 5. Correlation between SNF and monofilament testing. Proportion of patients with type 2 diabetes with improvement of at least 0.5 mm/fiber from baseline.

	10 g Changes					
	Improved	No Change	Worsened	Total	Measures of Association	
	N (%)	N (%)	N (%)	N	Spearman's	p-value
	Right foot difference from baseline				0.1961	0.0304
≥0.5 Improvement	20 (41.7)	28 (58.3)	0 (0.0)	48		
<0.5 Improvement	18 (24.3)	54 (73.0)	2 (2.7)	74		
Total	38 (31.2)	82 (67.2)	2 (1.6)	122		
	Left foot difference baseline				0.1037	0.2559
≥0.5 Improvement	17 (34.7)	32 (65.3)	0 (0.0)	42		
<0.5 Improvement	20 (27.4)	50 (68.5)	3 (4.1)	80		
Total	37 (30.3)	82 (67.2)	3 (2.5)	122		
	Dominant limb difference from baseline				0.1376	0.1499
≥0.5 Improvement	18 (39.1)	28 (60.9)	0 (0.0)	46		
<0.5 Improvement	18 (27.7)	45 (69.2)	2 (3.1)	65		
Total	36 (32.4)	73 (65.8)	2 (1.8)	111		
	Non-dominant limb difference from baseline				0.1243	0.1956
≥0.5 Improvement	17 (37.8)	28 (62.2)	0 (0.0)	45		
<0.5 Improvement	18 (27.7)	45 (69.2)	2 (3.1)	65		
Total	35 (31.8)	73 (66.4)	2 (1.8)	110		

4. Discussion

The results from this analysis confirm and extend previous findings with LMF-MC-PP in patients with diabetic neuropathy [22]-[24]. Importantly, results from this study demonstrated improvement over time in lower limb ENFD and by limb sensation assessed by the Semmes-Weinstein monofilament test. The mean improvement in ENFD over 6 months ranged from 0.6 to 1.1 fibers/mm. This improvement is smaller in comparison to the increase of 1.5 fibers/mm observed by Jacobs and Cheng; [27] however, that population was small (N = 11) with a lower baseline ENFD (mean 1.6 fibers/mm). The findings of the present study are likely representative of expected results with LMF-MC-PP in a population with less nerve degeneration at baseline. In a parallel study of 116 DPN patients followed for 2 years, the rate of ENFD change over time without intervention was estimated at −0.68 fibers/mm/year (Vinik, data on file).

The positive correlation between the objective ENFD measurement and the clinical sensation test, albeit statistically significant only in the right foot, reinforces the strength of the results through demonstrating that improvement on both features is happening in the same patient.

The rationale for the use of LMF-MC-PP in this population of patients with confirmed diabetic neuropathy is based on previously reported studies. In animal studies, the components of LMF-MC-PP have been shown to improve both endothelial function and nerve conduction [13]-[16] [18]. Results from smaller open-label studies of LMF-MC-PP in patients with diabetic neuropathy reported improvements in sensation, ENFD, and symptoms [23] [25]. In a prospective, double-blind, randomized study, the effects of LMF-MC-PP in 214 patients with diabetic neuropathy were evaluated [22]. After 24 weeks, a significant improvement in neuropathy symptoms was observed together with improvements in quality of life.

Limitations of this study include its observational design and lack of a parallel control group. However, the objective nature of the biopsy findings from ENFD, as the primary endpoint, adds credibility to the results. In addition, these findings are limited by the 6-month duration of follow up. Longer term studies are needed to more fully assess the effects of LMF-MC-PP on a slowly progressing disease such as diabetic neuropathy. No information on adverse events was systematically collected, but no serious adverse events were noted. In a controlled study of patients with type 2 diabetes and peripheral neuropathy, LMF-MC-PP was well tolerated with a low incidence of adverse events that were not significantly different from adverse events reported with placebo [22].

5. Conclusion

These results build on previous findings in patients with diabetic neuropathy that LMF-MC-PP provides improvement in objective and subjective measures of neuropathy. Larger, controlled studies evaluating LMF-MC-PP in patients with neuropathy that utilize objective measures of nerve function are warranted to confirm these findings and further establish the benefits of this therapy in patients with DPN.

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Conflict of Interest

This work was supported by grants from Nestlé Health Science-Pamlab, Inc, Covington, Louisiana. The sponsor participated in the study design, analysis, and review of the manuscript but was not involved in data collection.

Mrs. Barrentine is a full-time employee of Nestle Health Science-Pamlab, Inc. Research support for this study was provided to Dr. McNamara; he has no other conflicts of interest. Dr. De Vol received research support for this study; he has no other conflicts of interest. Dr. Vinik is a board member of Medscape. He serves as a consultant for Pfizer, Merck, Nestlé Health Science-Pamlab, Inc, Hydra Biosciences, Neurometrix, and ISIS Pharmaceuticals. Dr. Vinik has received research support from Pfizer, Impeto Medical Daiichi Sankyo Pharma, Tercica, Viromed, Intarcia Therapeutics, Vero Science, and Novo Nordisk. He also has received grants from the American Diabetes Association and from the National Institutes of Health. He is on the speaker's bureau for Merck and Nestlé Health Science-Pamlab, Inc.

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