Management of Diabetic Small-Fiber Neuropathy With Combination L-Methylfolate, Methylcobalamin, and Pyridoxal 5′-Phosphate

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Agents used to treat symptoms of diabetic peripheral neuropathy (DPN) are only palliative, not disease modifying. Although studies of monotherapy with L-methylfolate, methylcobalamin, or pyridoxal 5′-phosphate suggest that each of these bioavailable B vitamins may reverse the pathophysiology and symptoms of DPN, data on the efficacy of this combination therapy are limited. Therefore, we assessed the efficacy of an oral combination of L-methylfolate, methylcobalamin, and pyridoxal 5′-phosphate for improving epidermal nerve fiber density (ENFD) in the lower extremity of patients with DPN. Eleven consecutive patients with type 2 diabetes with symptomatic DPN were assessed for ENFD at the calf by means of skin punch biopsy and then placed on twice daily oral-combination L-methylfolate, methylcobalamin, and pyridoxal 5′-phosphate. After approximately 6 months of treatment, patients underwent follow-up biopsy. At the end of their treatment, 73% of patients showed an increase in calf ENFD, and 82% of patients experienced both reduced frequency and intensity of paresthesias and/or dysesthesias. This preliminary study suggests that combination L-methylfolate, methylcobalamin, and pyridoxal 5′-phosphate increases ENFD in patients with DPN.
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Key words: Diabetic peripheral neuropathy • Folate • Methylcobalamin • Pyridoxal 5′-phosphate • Epidermal nerve fiber density

The prevalence of diabetes mellitus (DM) in the United States population was about 7.8% in 2007.[1] Up to 60% of DM patients have diabetic peripheral neuropathy (DPN), a major cause of disability in the United States. DPN is primarily due to deterioration in small nerve fibers (myelinated A-δ and unmyelinated C-fibers) that mediate pain, temperature, and autonomic functions.[2-4] Neuropathy that is mainly or entirely associated with abnormalities of these fibers is termed a small-fiber neuropathy (SFN).

The most common causes of SFN are prediabetic states (such as impaired glucose tolerance) and frank diabetes. Although SFN is only one type of neuropathy affecting diabetic patients, it causes serious paresthesias, painful dysesthesias, and spontaneous pain.[5] Furthermore, lack of protective sensation of the feet occurs in as many as 30% of DM patients at least 40 years of age. Factors associated with worsening of SFN symptoms include patient age, duration of DM, and glycemic dysregulation.[6,7] Lack of protective sensation in diabetic patients puts them at high risk for foot ulcerations and nontraumatic amputations originating from undetected injury. Approximately 60% of all nontraumatic amputations performed in 2004 were on patients with DM.[1,8]

Lack of consensus regarding the underlying pathophysiology and optimal treatment of SFN has complicated its management.[9,10] Currently, agents used to reduce symptoms of pain and painful dysesthesias of SFN include anticonvulsants, tricyclic antidepressants, selective serotonin reuptake inhibitors, serotonin-norepinephrine reuptake inhibitors, and opioid or opioid-like analgesics. Among these medications, only duloxetine and pregabalin have been approved by the US Food and Drug Administration (FDA) for treatment of these symptoms. Unfortunately, there is no evidence that any of these agents modify the underlying pathophysiology of SFN. Thus, these medications exhibit merely palliative activity for SFN. Clearly, there is a need for agents that modify the underlying pathophysiology of DPN and, thus, SFN.[11]

Despite the lack of certainty about the pathophysiology of SFN, oxidative stress and nerve perfusion deficits appear to play important roles in it. One model proposes that hyperglycemia-induced oxidative stress in diabetic patients causes a depletion of vasodilating nitric oxide (NO), which, in turn, causes "endothelial dysfunction" and, consequentially, hypoperfusion of the microvasculature supplying nerves and peripheral nerve trunks. This hypoperfusion is believed to promote neuropathy and patient vulnerability to undetected injury, foot ulceration, and resultant lower extremity amputation.[11]

The theory that the pathogenesis of SFN involves oxidative stress and reduced nerve perfusion led to interest in L-methylfolate, methylcobalamin, and pyridoxal 5′-phosphate (metabolically active forms of folate, B_{12}, and B_6, respectively) as potential disease-modifying agents in SFN. Limited evidence suggests that L-methylfolate relieves endothelial dysfunction in patients with type 2 DM by improving the activity of endothelial NO synthase (eNOS) and, thus, the bioavailability of NO.[12] Also, L-methylfolate may eliminate products of oxidative stress, such as free radicals, including superoxide anion and peroxynitrite.[13] Orally administered methylcobalamin appears to promote myelin node genesis and nerve regeneration and reduce paresthesias and dysesthesias in SFN.[14,15] Pyridoxal deficiency has been shown to be associated with symptomatic SFN.[16]

Improvement in epidermal nerve fiber density (ENFD), assessed using the skin punch biopsy method developed by Polydefkis and colleagues,[3] is a surrogate marker of improvement of DPN or, more narrowly, SFN. This highly sensitive and reliable method of measuring ENFD to assess severity of SFN and response to treatment was an ideal technique for this study. The skin punch biopsy method, which involves direct quantification of pathologic changes in epidermal nerve fibers, is useful in identifying SFN due to various causes, including impaired glucose tolerance.[17-19]

We hypothesized that an orally administered combination of L-methylfolate, methylcobalamin, and pyridoxal 5′-phosphate (LMF-MC-PP) improves ENFD and, thus, SFN, in symptomatic type 2 DM patients with established SFN. The current clinical investigation was a case series of SFN patients in which immunohistochemical analysis of specimens obtained via skin punch biopsy was used to evaluate SFN and its response to treatment.

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Patients and Methods

Patients

Eleven consecutive patients with confirmed type 2 DM, as well as symptoms consistent with SFN of the feet, were recruited from the private practice of Dr. Jacobs. Verbal

consent was obtained from each patient after the study, procedure, and possible complications were described in detail. The study was conducted between July 2008 and March 2009.

Inclusion criteria consisted of diabetes with a history of both positive and negative sensory symptoms (eg, paresthesias, spontaneous pain, or dysesthesias) of the lower extremities. Exclusion criteria included history of hereditary neuropathy, use of any medication for diabetic neuropathy, and presence of any type of peripheral neuropathy that was not due to diabetes.

Information regarding symptoms, duration of diabetes, current medications, and comorbid conditions was obtained from each patient. Each patient reported symptoms consistent with SFN, such as numbness, burning, tingling, cramping, or weakness of the lower extremities. Each patient rated these symptoms at both onset and completion of treatment using a visual analog scale (VAS). Also, patients underwent a full baseline physical examination that included assessment of sensitivity to vibration, light touch, and cold temperature, as well as response to 10-g monofilament.

Methods

Consecutive type 2 DM patients with symptomatic DPN were assessed for ENFD at the calf by means of skin punch biopsy and then placed on twice daily oral-combination L-methylfolate (3 mg), methylcobalamin (2 mg), and pyridoxal 5′-phosphate (35 mg) for approximately 6 months. Patients then underwent follow-up biopsy (Table 1).

The standard biopsy protocol involved sterilizing the biopsy site with povidone-iodine and anesthetizing the site with 3 mL of injectable 0.5% bupivacaine hydrochloride with epinephrine 1:200,000. During the biopsy procedure, two 3-mm cutaneous punch biopsies (separated by a distance of about 5 mm) were obtained from a site 10 cm proximal to the lateral malleolus between the peroneal and Achilles tendons at a calf of each patient. Specimens were preserved in a standard fixative solution consisting of 2% periodate lysine paraformaldehyde and then sent for histologic analysis to Therapath, LLC (New York, NY).

The method of ENFD evaluation relied on immunohistochemical localization of a neural antigen within axons. Tissue sections from the 3-mm punch biopsy specimens were cut to 50 μm in thickness and stained with polyclonal antibodies recognizing the protein gene product (PGP) 9.5, which is present in all nerve fibers in the skin. To obtain the ENFD in fibers per millimeter (fibers/mm) of epidermis, pathologists manually counted the number of epidermal nerve fibers in three to five sections and divided the value by the sum of the lengths of the epidermal specimens in millimeters.[20] Pathologists were blinded to whether biopsy specimens were obtained before or after treatment. The primary efficacy endpoint was improvement in ENFD after approximately 6 months of treatment.

Table 1
Patient Characteristics

Patient #	Age of Patients (y)	Sex	Duration of Diabetes Mellitus (y)
1	73	F	8
2	52	M	20+
3	68	M	20+
4	53	M	10
5	51	F	2
6	41	F	10
7	75	F	< 0.5
8	63	F	20+
9	84	F	10
10	51	F	20+
11	54	M	2

Statistical Analysis

Statistical analysis was conducted by Dunlei Cheng, PhD, using SAS® 9.2 (SAS Software, Cary, NC). The research hypothesis was that LMF-MC-PP increases ENFD among study patients with SFN. A Wilcoxon signed-rank test was used to determine whether, for the average patient, ENFD increased significantly after 6 months of treatment with LMF-MC-PP. Spearman correlation based on rank was used to determine whether a difference in fiber density was related to age, sex, or duration of diabetes. Results were expressed in mean change in ENFD from baseline over the 6-month interval, as well as *P* value. In this study, a *P* value less than 0.05 was considered statistically significant.

Results

Among the 11 patients (4 men, 7 women, aged 41-84 years), duration of type 2 DM ranged from < 6 months to > 20 years (Table 1). At baseline, each of the 11 patients underwent two ENFD punch biopsies and was then placed on one oral tablet of combination LMF-MC-PP bid for approximately 6 months (median 5.7 ± 0.6 months). Although two post-treatment punch biopsies were obtained from 10 of the patients, only one post-treatment punch biopsy was obtained from the remaining patient (patient number 9).

Oral-combination LMF-MC-PP was well tolerated and no patient reported any adverse events. There were no adverse events due to biopsy.

Table 2
Duration of Treatment, and Average Baseline, Post-Treatment, and Increase in ENFD Values

Patient #	Duration of Treatment (mo)	Average Baseline ENFD (fibers/mm)	Average Post-Treatment ENFD (fibers/mm)	Average Increase in ENFD (fibers/mm)
1	5.7	3.86	7.1	3.24
2	5.8	0	3.76	3.76
3	5.1	0.17	2.05	1.88
4	5.7	0.26	0.35	0.09
5	5.6	4.62	4.93	0.31
6	5.2	0	0	0
7	5.7	4.99	6.71	1.72
8	5.2	1.63	5.38	3.75
9	5.6	1.26	2.63[a]	1.37[a]
10	6.1	0.28	0.66	0.38
11	6.1	0	0	0

[a]A second sample for ENFD was not obtained.
ENFD, epidermal nerve fiber density.

Change in ENFD and Symptoms After 6 Months of Treatment

ENFD was assessed by immunohistochemical staining of biopsy samples. The values for average baseline, post-treatment, and increase in ENFD in fibers/mm for each of the 11 patients are shown in Table 2 and illustrated in Figure 1. The mean ENFD of the 11 participants was 1.56 fibers/mm at baseline and 3.07 fibers/mm after approximately 6 months of oral treatment with combination LMF-MC-PP, representing a 97% increase in ENFD (P = .004) (Figure 2). Eight of the 11 (73%) patients experienced an increase in ENFD during an approximately 6-month course of treatment with LMF-MC-PP (Figure 1). The mean per-patient increase in ENFD was 1.5 fibers/mm.

Eight of the 11 (73%) patients experienced an increase in ENFD during an approximately 6-month course of treatment with LMF-MC-PP.

Comparison of immunohistochemically stained sections of epidermis revealed improvements after approximately 6 months of treatment with combination LMF-MC-PP. The photomicrographs in Figure 3 show immunohistochemically stained baseline and post-treatment sections of epidermis from patient 8. The post-treatment section shows regenerating small nerve fibers in both the basement membrane and keratin layers. Based on ENFD measurement, the patient experienced a mean increase of 3.75 fibers/mm during approximately 6 months of treatment. A total of 82% of study patients reported reduced frequency and intensity of paresthesias and dysesthesias after 6 months of treatment.

Statistical Analysis of Changes in ENFD After Treatment

For the changes in ENFD, the Wilcoxon test statistic was 22.5, with a derived P value of 0.004, which is much smaller than a conventional type I error rate of 0.05. This finding indicated that, for the average patient in the study, the increase in ENFD after approximately 6 months of treatment with oral-combination LMF-MC-PP was statistically significant. The Spearman correlations were 0.30 (P = .40) between increase in ENFD and age; − 0.36 (P = .28) between increase in ENFD and sex, favoring female sex (though not significantly); and 0.75 (P = .02) between increase in ENFD and duration of diabetic history. Thus, only duration of diabetes correlated significantly with increase in ENFD after therapy with LMF-MC-PP, implying that a longer duration of diabetes might be associated with a greater increase in ENFD after treatment.

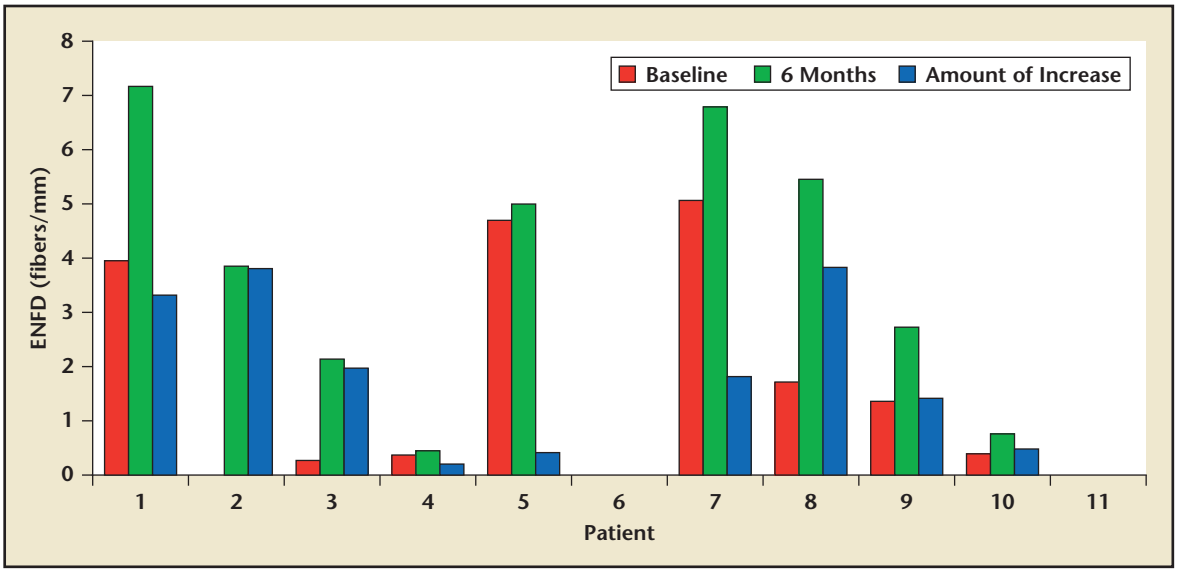

Figure 1. *Eight of the 11 patients (73%) experienced an increase in ENFD during an approximately 6-month course of treatment with the oral combination LMF-MC-PP. For patient 2, the baseline ENFD value was zero. For patients 6 and 11, both baseline and post-treatment ENFD values were zero. For patient 9, the baseline value was based on one biopsy. ENFD, epidermal nerve fiber density; LMF-MC-PP, L-methylfolate, methylcobalamin, and pyridoxal 5'-phosphate.*

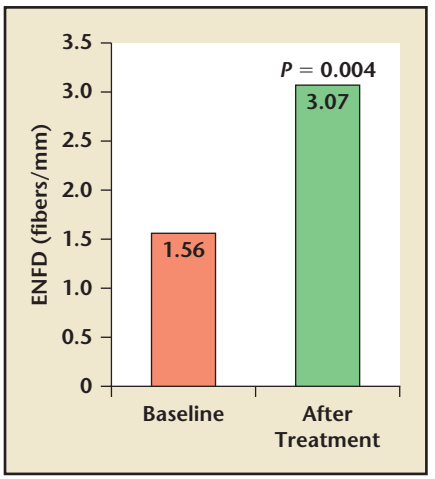

Figure 2. *The mean ENFD of the 11 patients was 1.56/fibers/mm at baseline and 3.07/ fibers/mm after approximately 6 months of treatment with oral-combination LMF-MC-PP, representing a 97% increase in ENFD (P = .004). ENFD, epidermal nerve fiber density; LMF-MC-PP, L-methylfolate, methylcobalamin, and pyridoxal 5'-phosphate.*

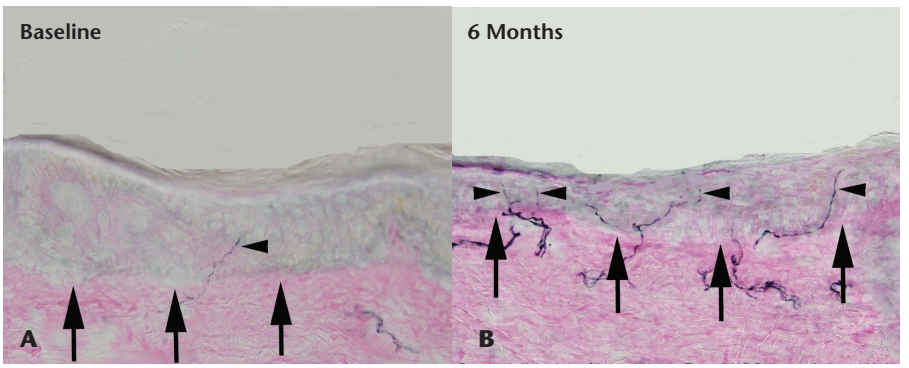

Figure 3. *Photomicrographs of immunohistochemically stained sections of epidermis from the left calf of patient 8, who had type 2 diabetes and diabetic small-fiber neuropathy and was treated with combination LMF-MC-PP twice daily for 6 months.* **(A)** *Photomicrograph of baseline skin punch biopsy taken from the left calf revealed low ENFD. The basement membrane is indicated by three vertical arrows. An intraepidermal nerve fiber in the keratin layer is indicated by a horizontal triangle.* **(B)** *Photomicrograph of a similar sample from the same patient taken after approximately 6 months of treatment. Based on measurement of ENFD, a mean increase of 3.75 nerve fibers/mm had occurred during treatment. Regenerating small nerve fibers can be seen in both the basement membrane (indicated by four* vertical arrows*) and keratin layer (indicated by* horizontal triangles*). Original magnification x 20. ENFD, epidermal nerve fiber density; LMF-MC-PP, L-methylfolate, methylcobalamin, and pyridoxal 5'-phosphate. Image courtesy of Therapath, LLC (New York, NY). Reproduction is prohibited without written consent of Therapath.*

Discussion

Potential Benefits of LMF-MC-PP Therapy in Patients With SFN

This is the first clinical study to suggest that treatment with LMF-MC-PP may promote statistically significant improvement in ENFD in patients with SFN. The finding that this improvement in ENFD was associated with decreased anesthesia, paresthesia, or dysesthesia in greater than 80% of study patients implies that improvement in these symptoms was due to increased ENFD.

These results were consistent with those of a 2009 pilot study by Walker and colleagues[21] in which a series of type 2 diabetic patients with SFN who received LMF-MC-PP for 1 year experienced progressive and statistically significant improvement in cutaneous sensitivity of the feet, as measured by one- and two-point static Pressure-Specified Sensory Device testing. Together, these two studies

provide evidence that LMF-MC-PP promotes restoration of damaged cutaneous nerve fibers in patients with SFN.

The findings of the current study also are consistent with the theory that treatment with medical nerve foods that promote the bioavailability of endothelial NO may alter the underlying pathogenesis of DPN and consequently SFN. As noted earlier, evidence indicates that each of the components of LMF-MC-PP may have the potential to improve SFN by modifying its underlying pathophysiology. Both pyridoxal 5′-phosphate and methylcobalamin monotherapy may be essential to critical peripheral nerve functions that are impaired in DPN.[14,15,22] L-methylfolate has been shown to improve endothelial function in patients with type 2 DM, possibly by promoting synthesis of NO.[12,13,23]

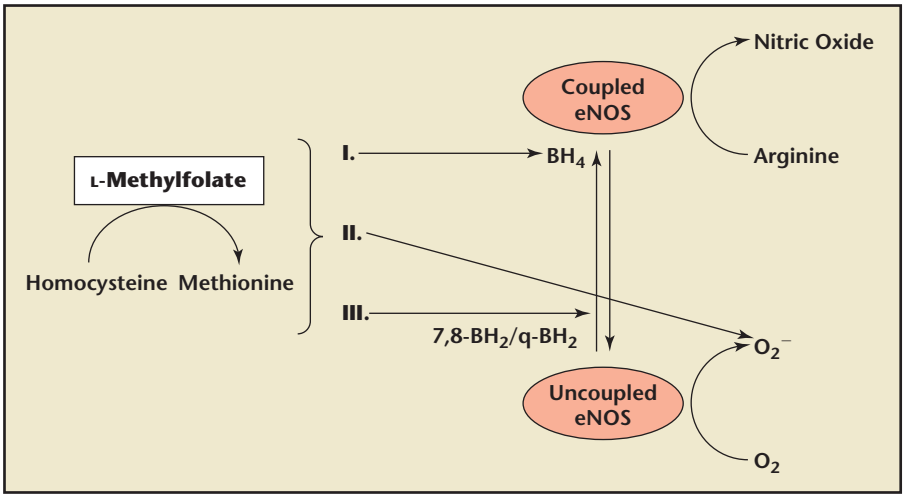

Figure 4. *Nitric oxide synthesis and potential mechanisms of action of L-methylfolate. Diagram illustrates nitric oxide (NO) synthesis from L-arginine, as catalyzed by endothelial NO synthase (eNOS), and potential mechanisms for beneficial effects of L-methylfolate on eNOS. The beneficial effects of L-methylfolate may be explained by different mechanisms. I. Tetrahydrobiopterin (BH_4) rescue or BH_4 stabilization, in which L-methylfolate may stimulate endogenous BH_4 regeneration from quinoid-dihydrobiopterin ($q\text{-}BH_2$) or lead to chemical stabilization of BH_4. II. Antioxidant effects, in which L-methylfolate may act as a direct antioxidant. III. Direct effect on eNOS, in which L-methylfolate reduces superoxide generation and increases NO synthesis in a BH_4-dependent manner. Adapted with permission from Verhaar MC, Stroes E, Rabelink TJ. Folates and cardiovascular disease.* Arterioscler Thromb Vasc Biol. *2002;22:6-11.*[13]

Pathogenesis of SFN

The pathogenesis of SFN appears to be multifaceted and attributable to the effects of hyperglycemia, oxidative stress, mitochondrial dysfunction, and altered transport of nutrients within nerve axons as a result of accumulation of advanced glycation end products.[24,25] Oxidative stress involves uncoupling the enzyme eNOS in diabetic patients and thus interferes with endothelial production of NO, a gaseous free radical that mediates relaxation of vascular smooth muscle and promotes tissue repair.[26] As is depicted in Figure 4, reduction in NO results in constriction of blood vessels, reduced capillary flow, nerve hypoxia, and, over time, SFN.[12,13,22,27] Interestingly, reduction in NO synthesis has also been shown to be associated with impaired wound healing in diabetic patients.[11,27,28] Histologic findings in SFN include reduced cutaneous peripheral nerve fiber branch density and length, dermal and epidermal axon swelling, subepidermal nerve plexus thinning, nerve terminal sprouting and encapsulation, and presence of mural reactive basal cells.[29-31]

Another clue to the pathogenesis of SFN comes from studies showing that elevated homocysteine, which is often present in diabetic patients with neuropathy, may be a risk factor for DPN in general, including autonomic neuropathy.[7,32,33] One widely held theory is that hyperhomocysteinemia is directly toxic to the vascular endothelium and induces an endothelial dysfunction that promotes endothelial thrombosis and, consequently, reduced perfusion of peripheral nerves.[34] Another theory is that hyperhomocysteinemia indirectly promotes SFN by disrupting endothelial synthesis of NO, a smooth muscle relaxant that facilitates blood flow to peripheral nerves.[35]

Evidence that L-methylfolate, methylcobalamin, and pyridoxine 5′phosphate individually reduce homocysteine and promote NO synthesis suggests that these B vitamins have the capacity to alleviate both positive and negative symptoms of diabetic neuropathy. L-methylfolate, the metabolically active form of folic acid, is a cofactor for homocysteine metabolism in that it provides a methyl group to homocysteine for reconversion to methionine[36]; moreover, L-methylfolate directly promotes

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increased endothelial production of NO.[12,13]

L-methylfolate in oral-combination LMF-MC-PP offers several advantages over folic acid as a source of folate. First, the biologic activity of L-methylfolate is seven times that of folic acid. Second, although folic acid must undergo a complex four-step conversion process to become activated, L-methylfolate already is metabolically active. Finally, metabolically active L-methylfolate benefits those patients who carry a genetic polymorphism that prevents their converting folic acid to L-methylfolate.[34,37]

The formulation of LMF-MC-PP used in this study (Metanx®; Pamlab, Covington, LA) is classified by the FDA as a prescription medical food. The FDA defines a medical food as a substance that is prescribed by a physician "for the specific dietary management of a disease or condition for which distinctive nutritional requirements, based on recognized scientific principles, are established by medical evaluation."[38] LMF-MC-PP use is indicated for the distinct nutritional requirements of diabetic patients with endothelial dysfunction[12,39,40] who present with loss of protective sensation and neuropathic pain[41,42] associated with diabetic peripheral neuropathy.[43]

Reliable Method for Diagnosing SFN

Currently, the most reliable approach to diagnosing SFN involves demonstrating either reduction in number or altered morphology of epidermal nerve fibers by means of skin punch biopsy.[44-48] Direct evaluation of ENFD in skin obtained by skin punch biopsy is a more sensitive method for diagnosing SFN than use of sensory nerve conduction studies alone.[6,47,49,50] Although the sensitivities of clinical examination and quantitative sensory thermal threshold testing for diagnosing SFN are only 54% and 49%, respectively, the sensitivity of skin punch biopsy in diagnosing SFN is up to 88.4%. The specificity of skin punch biopsy in diagnosing SFN is as high as 97%.[51,52]

Determination of ENFD by skin punch biopsy has a broad range of applications, including differentiation among distal polyneuropathy, sensory radiculopathy, and focal neuropathy. The technique is useful in detecting other neuropathies, such as those related to vasculitis, amyloid, or sarcoid, as well as monitoring progression of neuropathy or response to treatment.[51] Combining quantitative sensory testing with ENFD determination by skin punch biopsy is effective in diagnosing suspected SFN in diabetic or glucose-intolerant patients with neuropathic pain, even prior to the development of signs of either autonomic small-fiber or sensory large-fiber pathology.[4]

Assessing Theoretical Limitations and Advantages of Study

The current study was limited by its small size, method of participant selection, possible volunteer bias, lack of a placebo-treated group for comparison, lack of blinding, and the subjective nature of the VAS assessment. Also, the study did not control for differences in duration of type 2 DM among participants and the possibility that only some patients used insulin and/or a symptom-reducing medication. Despite these limitations, the finding of statistically significant changes in ENFD based on a highly valid, objective method of testing is compelling.

Main Points

- As many as 60% of patients with diabetes mellitus have small-fiber peripheral neuropathy (SFN), a leading cause of disability in the form of paresthesias, painful dysesthesias, and lack of protective sensation, which puts these patients at high risk for foot ulcerations and nontraumatic amputations originating from undetected injury.
- There is no evidence that agents currently used to reduce paresthesias and dysesthesias of SFN modify the underlying pathophysiology of SFN; these medications appear to have only palliative activity in SFN.
- Studies of monotherapy with L-methylfolate, methylcobalamin, or pyridoxal 5′-phosphate suggest that these B vitamins may reverse both the pathophysiology and symptoms of diabetic SFN.
- We hypothesized that oral combination L-methylfolate, 3 mg, methylcobalamin, 2 mg, and pyridoxal 5′-phosphate, 35 mg (LMF-MC-PP), improves epidermal nerve fiber density (as determined by immunohistochemical analysis of skin punch biopsy), in symptomatic patients with type 2 diabetes and established SFN.
- Our study found that treatment with LMF-MC-PP was associated with a statistically significant increase in epidermal nerve fiber density and a reduction in frequency and intensity of paresthesias and dysesthesias.
- The data from this pilot study involving a combination of metabolically active LMF-MC-PP are consistent with studies of monotherapy with these B vitamins and suggests that LMF-MC-PP modifies the pathophysiology of diabetic SFN and improves perfusion and thus sensory perception of peripheral nerves in patients with diabetic SFN.

Conclusions

This study suggests that oral administration of LMF-MC-PP promotes increase in ENFD in participants with diabetic SFN and improves symptoms of anesthesia, paresthesia, or dysesthesia. A high-quality, double-blinded, randomized, controlled trial is necessary to confirm the effectiveness of LMF-MC-PP in SFN. Meanwhile, LMF-MC-PP should be considered in the treatment of patients with diabetic SFN in whom other therapies have been ineffective. ■

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