Journal of Hainan Medical University

http://www.hnykdxxb.com

Effect of mNGF injection combined with vitamin D and methylamine on neurological function and oxidative stress in patients with diabetic peripheral neuropathy

Guo Li✉

Pharmacy Department, Zigong Third People's Hospital in Sichuan Province, Zigong City, Sichuan Province, 643020

ARTICLE INFO

Article history:
Received 6 Jun 2017
Received in revised form 10 Jun 2017
Accepted 16 Jun 2017
Available online 28 Jun 2017

Keywords:
Diabetic peripheral neuropathy
mNGF
Neurological function
Oxidation stress

ABSTRACT

Objective: To study the effect of mNGF injection combined with vitamin D and methylamine on neurological function and oxidative stress in patients with diabetic peripheral neuropathy. **Methods:** 120 patients with diabetic peripheral neuropathy who were treated in our hospital between July 2012 and February 2016 were collected and randomly divided into control group (n=60) and observation group (n=60), control group were treated with vitamin D and methylamine, observation group received mNGF injection combined with vitamin D and methylamine therapy, and both therapies lasted for 8 weeks. Electromyography indexes, neurological function indexes and oxidative stress indexes were compared between two groups of patients before and after treatment. **Results:** Before treatment, the differences in electromyography indexes, neurological function indexes and oxidative stress indexes were not statistically significant between two groups of patients. After treatment, the MCV and SCV levels of median nerve, ulnar nerve and tibial nerve in observation group were higher than those in control group; serum neurological function indexes BDNF and IGF-1 contents were higher than those in control group while MBP content was lower than that in control group; serum oxidative stress indexes SOD, GSH and T-AOC contents were higher than those in control group while MDA and AOPP contents were lower than those in control group. **Conclusion:** mNGF injection combined with vitamin D and methylamine can optimize the electromyography and neurological function, and reduce the systemic oxidation stress response in patients with DPN.

1. Introduction

Diabetic peripheral neuropathy (DPN) refers to the peripheral nerve sensory and motor disorders in patients with diabetes, it is caused by glucose and lipid metabolism disorder, vascular injury, insufficient neurotrophic factors and multiple other factors, and severe cases can lead to serious decline in daily behavior ability and severe pain, and bring great trouble to the patient's normal life[1,2]. Vitamin D and mecobalamine are both common drugs for DPN treatment, they reduce insulin resistance and optimize the damaged nerve cell function to alleviate the clinical symptoms of patients with DPN, but the overall efficacy is limited[3]. Mouse nerve growth factor (mNGF) can accelerate the neurotransmitter synthesis in damaged nerve and promote the regeneration of neurons, and it has been successfully applied in the treatment of cerebral stroke, cerebral hemorrhage and other neurological disorders[4,5]. In this study, mNGF was added to the DPN therapy, and the effects of comprehensive treatment on the neurological function, oxidative stress and other aspects were explored, hereby reported as follows.

2. Information and methods

2.1 Case information

120 patients with diabetic peripheral neuropathy who were treated in our hospital between July 2012 and February 2016 were collected as research subjects, and patients themselves signed the

✉Corresponding author: Guo Li, Pharmacy Department, Zigong Third People's Hospital in Sichuan Province, Zigong City, Sichuan Province, 643020
Tel: 0813-3312034; 13909001692
Fund Project: Project of Zigong Science and Technology Bureau No: 2015S07.

informed consent. According to random number table, the enrolled patients were divided into control group (n=60) and observation group (n=60). Control group included 34 male cases and 26 female cases, they were 48-79 years old, the course of diabetes was 7-20 years, and the course of DPN is 6 months-4 years; observation group included 33 male cases and 27 female cases, they were 46-78 years old, the course of diabetes was 8-19 years, and the course of DPN is 5 months-3 years. The distribution of gender, age, course of diabetes and course of DPN were not statistically different between the two groups of patients (P>0.05). The study was approved by the ethics committee of the hospital.

2.2 Inclusion and exclusion criteria

Inclusion criteria: (1) meeting the diagnostic criteria for DPN in the 2013 guidelines for diabetes prevention in China; (2) receiving systemic therapy for the first time; (3) cooperating with all the treatments and inspections. Exclusion criteria: (1) with allergies of mNGF injection, vitamin D, mecobalamine and so on; (2) associated with severe heart, liver and kidney insufficiency; (3) associated with systemic infectious diseases; (4) associated with malignant tumor disease.

2.3 Therapy

Both groups of patients received routine blood sugar control therapy, and control group received vitamin D and mecobalamine treatment on the basis of routine hypoglycemic treatment, specifically as follows: vitamin D tablets (Sinopharm Xingsha Pharmaceutical Co., Ltd., approved by H35021450), taken orally, 1 tablet/time, 1 time/d; methylamine (Beijing Sunho Pharmaceutical Co., Ltd., approved by H20060865) 0.5 mg/time, 3 times/d, for continuous 8 weeks of treatment. Observation group of patients received mNGF injection treatment on the basis of the treatment of control group, specifically as follows: mNGF injection (Livzon Group Livzon Pharmaceutical Factory, approved by S20100005), by intramuscular injection, 30 μg/time, 1 time/d, for continuous 8 weeks of treatment.

2.4 Observation indexes

Before and after treatment, affected limb electromyography of two groups of patients were determined, including the motor conduction velocity (MCV) and sensory conduction velocity (SCV) of the median nerve, ulnar nerve and tibial nerve, the room temperature of examination room was controlled at 25 ℃, and the indexes were measured for 3 times in a row to take the averaging. Proper amount of fasting cubital venous blood was extracted from two groups of patients at the same points in time, anti-coagulated and then centrifuged at low speed to get upper serum, and enzyme-linked immunosorbent assay (ELISA) was used to determine the contents of neurological function indexes and oxidative stress indexes, including the neurological function-related parameters brain-derived neurotrophic factor (BDNF), myelin basic protein (MBP) and insulin-like growth factor-1 (IGF-1) as well as oxidative stress indexes superoxide dismutase (SOD), glutathione peroxidase (GSH), total antioxidant capacity (T-AOC), malondialdehyde (MDA) and advanced oxidation protein products (AOPP).

2.5 Statistical processing

Data in the study were recorded and analyzed by specially-assigned person, and statistical software was SPSS 20.0. Electromyography indexes, neurological function indexes, oxidative stress indexes and other measurement data were in terms of mean ± standard deviation, and comparison was by t test. P<0.05 was the standard of statistical significance in differences within group and between groups.

3. Results

3.1 Electromyography indexes

Comparison of the MCV and SCV levels of the median nerve, ulnar nerve and tibial nerve between two groups of patients before and after treatment was as follows: before treatment, the differences in the MCV and SCV levels of median nerve, ulnar nerve and tibial nerve were not statistically significant between two groups of patients (P>0.05); after treatment, the MCV and SCV levels of median nerve, ulnar nerve and tibial nerve in both groups were significantly higher than those before treatment, the MCV and SCV levels of median nerve, ulnar nerve and tibial nerve in observation group were higher than those in control group, and differences were statistically significant (P<0.05), shown in Table 1.

3.2 Neurological function indexes

Before and after treatment, comparison of serum neurological function-related indexes BDNF (ng/mL), MBP (μg/L)and IGF-1 (pg/mL) contents between two groups of patients was as follows: before treatment, serum BDNF, MBP and IGF-1 contents were not significantly different between two groups of patients (P>0.05); after

Table 1.
Comparison of electromyography index levels between two groups of patients before and after treatment (m/s).

Groups	n	Time	Median nerve		Ulnar nerve		Tibial nerve	
			MCV	SCV	MCV	SCV	MCV	SCV
Control group	60	Before treatment	40.27±4.89	34.19±4.53	30.47±4.18	32.46±3.95	38.49±4.53	34.82±4.77
		After treatment	48.61±5.37[a]	45.26±5.28[a]	41.56±4.85[a]	40.52±4.88[a]	45.72±5.85[a]	43.17±5.48[a]
Observation group	60	Before treatment	40.53±4.94	34.23±4.47	30.62±4.09	32.51±3.88	38.64±4.37	34.69±4.62
		After treatment	53.78±6.19[ab]	55.62±6.84[ab]	50.83±6.72[ab]	49.82±6.14[ab]	53.19±6.43[ab]	51.33±5.98[ab]

Note: compared with same group before treatment, [a]P<0.05; compared with control group after treatment, [b]P<0.05.

Table 2.
Comparison of serum BDNF, MBP and IGF-1 contents before and after treatment.

Groups	*n*	Time	BDNF	MBP	IGF-1
Control group	60	Before treatment	3.18±0.45	0.94±0.11	3.28±0.46
		After treatment	6.27±0.77[a]	0.68±0.07[a]	5.11±0.63[a]
Observation group	60	Before treatment	3.20±0.43	0.92±0.14	3.26±0.43
		After treatment	11.54±1.89[ab]	0.37±0.05[ab]	9.07±1.18[ab]

Note: compared with same group before treatment, [a]$P<0.05$; compared with control group after treatment, [b]$P<0.05$.

Table 3.
Comparison of serum oxidative stress index contents before and after treatment.

Groups	*n*	Time	SOD	GSH	T-AOC	MDA	AOPP
Control group	60	Before treatment	75.38±9.17	134.28±15.93	5.48±0.73	26.58±3.41	11.24±1.79
		After treatment	92.17±10.24[a]	171.53±20.34[a]	8.29±0.95[a]	18.34±2.15[a]	8.63±1.17[a]
Observation group	60	Before treatment	75.47±9.05	133.76±15.78	5.51±0.72	26.49±3.52	11.35±1.89
		After treatment	157.48±18.94[ab]	215.47±25.83[ab]	13.43±1.85[ab]	11.62±1.85[ab]	3.75±1.28[ab]

Note: compared with same group before treatment, [a]$P<0.05$; compared with control group after treatment, [b]$P<0.05$.

treatment, serum BDNF and IGF-1 contents in both groups were significantly higher than those before treatment while MBP contents were lower than those before treatment; serum BDNF and IGF-1 contents in observation group were significantly higher than those in control group while MBP content was lower than that in control group ($P<0.05$), shown in Table 2.

3.3 Oxidative stress indexes

Before and after treatment, comparison of serum oxidative stress indexes SOD (mg/L), GSH (mg/L), T-AOC (U/mL), MDA (nmol/L) and AOPP (ng/mL) contents between two groups of patients was as follows: before treatment, serum SOD, GSH, T-AOC, MDA and AOPP contents were not significantly different between two groups of patients ($P>0.05$); after treatment, serum SOD, GSH and T-AOC contents in both groups were higher than those before treatment while MDA and AOPP contents were lower than those before treatment; compared with those in control group, serum SOD, GSH and T-AOC contents in observation group increased significantly while MDA and AOPP contents decreased significantly ($P<0.05$), shown in Table 3.

4. Discussion

DPN is one of the serious complications of diabetic patients with poor blood sugar control, and in addition to routine hypoglycemic treatment, repairing the nerve function is the main method to reverse the disease[6,7]. Vitamin D belongs to the steroid hormone, which not only plays the basic roles such as maintaining calcium phosphorus metabolism and balance, but can also promote insulin secretion and relieve insulin resistance so as to optimize the glucose metabolism. Mecobalamine is the recognized drug for treatment of peripheral nerve injury, it is the derivative of vitamin B12, it can act on the damaged nerve to promote axoplasm protein synthesis, and the efficacy of early repair of damaged nerve is obvious, but its role is gradually weakened with the extension of treatment cycle[8]. Vitamin D combined with mecobalamine is the conventional scheme for DPN treatment at present, the medicinal properties and overall efficacy have limitations, and therefore, other targeted drug are needed to expand the curative effect. mNGF is extracted from mouse submandibular gland, the homology to human nerve growth factor is more than 95%, glucose metabolism disorder leads to the decline in nerve growth factor levels, and so the supplementation of exogenous nerve growth factor is the reliable method to cure the disease[9,10]. At present, there are many reports about the effectiveness of mNGF in treating central nerve injury diseases, but its application value for peripheral neuropathy is less covered. In the research, mNGF was used as auxiliary medicine to treat patients with DPN, and its clinical application value was confirmed and provided practical basis for the choice of subsequent clinical treatment options for same cases.

DPN patients are mainly characterized by slowing of sensory and motor conduction velocity, the median nerve, ulnar nerve and tibial nerve are the most commonly involved, and their nerve conduction velocity can objectively reflect the DPN severity[11,12]. In the study, the sensory and motor conduction velocity of theses nerves before and after treatment were compared between two groups of patients at first, and it was found that the MCV and SCV levels of median nerve, ulnar nerve and tibial nerve of both groups of patients increased after treatment, confirming that both therapies can optimize the function of damaged peripheral nerve. Further comparison showed that the MCV and SCV levels of median nerve, ulnar nerve and tibial nerve in observation group were higher than those in control group after treatment, indicating that adding mNGF treatment can further promote the repair of damaged nerve function, and macroscopically confirming that adjuvant mNGF therapy can improve neural function in patients with DPN.

The lack of nerve growth factor is the main cause of DPN occurrence and development, and also the fundamental mechanism of the decrease of MCV and SCV levels, BDNF, MBP and IGF-1 are all factors that are closely related to nerve nutrition and growth, and their content fluctuations are closely related to the patient' condition changes[13]. BDNF is a protein synthesized in the brain, it plays an important role for the neuron survival, growth and development, and can improve the physiological state of the neurons and inhibit the neuron apoptosis, and research has suggested that BDNF expression decreases in circulating blood of patients with DPN, which reduces

the protective effect on peripheral nerve[14]. MBP is the main protein of myelin sheath, it is nerve tissue-specific, it is released into the blood and detected in the case of neuron damage, and therefore, high level of MBP is a sign of serious nerve damage. IGF-1 is an insulin-like agent that is synthesized and secreted by the liver, which promotes the uptake of glucose by tissue and increases the growth and differentiation of the neurons[5]. In the study, the contents of these neurological function indexes were compared between two groups of patients, and it was found that serum BDNF and IGF-1 contents in both groups after treatment were higher than those before treatment while MBP contents were lower than those before treatment, which confirms the reliability of both therapies, and meanwhile, serum BDNF and IGF-1 contents in observation group after treatment were higher than those in control group while MBP content was lower than that in control group, which explains that adjuvant mNGF therapy can help increase the nerve nutrients and optimize the neural function.

Continuous high blood glucose can activate the patient's oxidative stress state, also make the damaged peripheral nerve further lose nutritional support and ultimately lead to DPN[15]. Previous studies have shown that antioxidants can be effective in alleviating peripheral neurological functional impairment in patients with DNP, and confirmed that oxidative stress response plays an important role in the development of DNP. SOD, GSH and T-AOC are the antioxidant factors, SOD and GSH catalyze reduction reaction to remove oxygen free radicals and reduce oxidative stress, and T-AOC reflects the strength of the overall antioxidant effect; MDA and AOPP are the representative oxidation products, the former is the product of lipid oxidation reaction, the latter is the product of protein oxidation reaction, and they can reflect the degree of oxidative stress reaction[16]. It was found in the study that antioxidant factors SOD, GSH, and T-AOC contents in both groups after treatment were higher than those before treatment while oxidative stress products MDA and AOPP contents were lower than those before treatment; the changes in above index contents in observation group were more than those in control group, it confirms that adjuvant mNGF therapy can decrease the systemic oxidative stress response in patients with DNP, and this also is one of the important mechanisms for it to ultimately optimize neural function.

To sum up, it can be concluded that mNGF injection combined with vitamin D and mecobalamine can effectively improve the neurological function of patients with DNP and reduce the systemic inflammatory response, it is a reliable way to treat the disease, and it is worthy of popularization and application in clinical practice in the future.

References

[1] Ou Jue-zhang, Jia Xiang, Chen Shu-qun. Effect of cinepazide combined with mecobalamine on nerve conduction velocity, vibration perception threshold and serum indexes in patients with diabetic peripheral neuropathy. *J Hainan Med Univ* 2017; **23**(3): 349-352.

[2] Bailey A, Wingard D, Allison M, Summers P, Calac D. Acupuncture treatment of diabetic peripheral neuropathy in an american indian community. *J Acupunct Meridian Stud* 2017; **10**(2): 90-95.

[3] Shi Rui, Sun Pei, Wang Lulu, Ding Lin, Xia Jin, Wang Yan, et al. Vitamin D and mecobalamin combined with mouse nerve growth factor for diabetic peripheral neuropathy: a clinical observation. *J Shandong Univ* 2016; **54**(4): 64-67.

[4] Liu Yun-tao, Pan Jing-fang, Jian Lei. The changes of serum stromal cell-derived factor-1 in patients with diabetic peripheral neuropathy and the influence of mouse nerve growth factor. *Chin J Diabetes* 2015; **23**(7): 612-615.

[5] Li X, Zhang J, Zhao W, Yang H, Ma J, Qi Y, et al. Effect of Tongxinluo on nerve regeneration in mice with diabetic peripheral neuropathy. *Cell Mol Biol (Noisy–le–grand)* 2015; **61**(5): 103-107.

[6] Zhou Hao-ling, Yan Can-qun. Research advances in diabetic peripheral neuropathy. *J Mil Surg Southwest China* 2017; 1**9**(1): 81-84.

[7] Yang X, Yao W, Liu H, Gao Y, Liu R, Xu L. Tangluoning, a traditional Chinese medicine, attenuates in vivo and in vitro diabetic peripheral neuropathy through modulation of PERK/Nrf2 pathway. *Sci Rep* 2017; **7**(1): 1014.

[8] Pang B, Zhao TY, Zhao LH, Wan F, Ye R, Zhou Q, et al. Huangqi Guizhi Wuwu Decoction for treating diabetic peripheral neuropathy: a meta-analysis of 16 randomized controlled trials. *Neural Regen Res* 2016; 11(8): 1347-1358.

[9] Li Ya-nan, Mao Quan-gao, Shen Jin-hua. Comparison on the therapeutic effect of GM1and mNGF in the treatment of diabetic peripheral neuropathy. *J Qiqihar Univ Med* 2016; **37**(19): 2377-2380.

[10]Peng L, Liu W, Zhai F, He L, Wang H. Microvessel permeability correlates with diabetic peripheral neuropathy in early stage of streptozotocin-induced diabetes rats. *J Diabetes Complication* 2015; **29**(7): 865-871.

[11]Li Yu, Qi Ling, Li Jun. Effect of hyperbaric oxygen combined with α -lipoic acid on neurological function and serum indexes of patients with diabetic peripheral neuropathy. *J Hainan Med Univ* 2016; **22**(13): 1394-1397.

[12]Huang Yunfei, Huang Chun, Song Yuling, Luo Qiuyu, Liu Jie. Influence of epalrestat combined with alprostadil on neuropathy symptoms, blood vessel function, neurological function of patients with diabetic peripheral neuropathy. *China Modern Doctor* 2014; **52**(11): 31-34.

[13]Vinik AI, Perrot S, Vinik EJ, Pazdera L, Jacobs H, Stoker M, et al. Capsaicin 8% patch repeat treatment plus standard of care (SOC) versus SOC alone in painfuldiabetic peripheral neuropathy: a randomised, 52-week, open-label, safety study. *BMC Neurol* 2016; **16**(1): 251.

[14]Jin YP, Su XF, Li HQ, Wu JD, Ding B, Sun R, et al. The therapeutic effect of pancreatic kininogenase on treatment of diabetic peripheral neuropathy in patients with type 2 diabetes. *Exp Clin Endocrinol Diabetes* 2016; **124**(10): 618-621.

[15]Wei Jian-fen, Wu Nai-jun, Chen Dong, Wang Ying, Ma Shao-jie. The correlation between serum ferritin and oxidative stress response in patients with peripheral neuropathy. *Guangdong Med J* 2014; **35**(18): 2887-2890.

[16]Liu Jie, Sun Bing, Ban Bo, Xu Xuan, Zhang Mei, Sun Hailing, et al. Investigation the mechanism of oxidative stress in diabetic peripheral neuropathy. *Beijing Med J* 2015; **37**(1): 16-19.