

Animal Study

The Different Dynamic Changes of Nerve Growth Factor in the Dorsal Horn and Dorsal Root Ganglion Leads to Hyperalgesia and Allodynia in Diabetic Neuropathic Pain

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Background: Diabetic peripheral neuropathy (DPN) is the most common complication of diabetes and more than half of the patients with DPN have self-reported symptoms referring to painful diabetic neuropathy (PDN). Nerve growth factor (NGF) is a key factor for the nervous system, but the role of it in the neuropathic pain of diabetic patients is unclear.

Objective: This study aimed to investigate the relationship between the dynamic expression of NGF in dorsal horn and dorsal root ganglion (DRG) of diabetic rats and hyperalgesia and allodynia in diabetic neuropathic pain. It also aimed to explore the effects of exogenous mouse NGF (mNGF) on NGF expression in dorsal horn, DRG, and mechanical pain threshold.

Study Design: Animal research study.

Setting: Experimental research laboratory.

Methods: The model of diabetes was established by a single intraperitoneal injection of streptozocin (STZ 55 mg/kg). Firstly, the rats were randomly divided into 2 groups: control group (n = 10) and diabetes group (n = 40). The diabetes group contained 4 subgroups: diabetes week 1 group (DM1, n = 10), diabetes week 2 group (DM2, n = 10), diabetes week 4 group (DM4, n = 10), and diabetes week 8 group (DM8, n = 10). Then, the other rats were randomly divided into 2 groups: control group (n = 10) and treatment group (n = 30). The treatment group contained 3 subgroups: saline group (n = 10), low dose mNGF group (mNGF1, n = 10), and high dose mNGF group (mNGF2, n = 10). Mechanical pain threshold was assessed using Von Frey hairs, before the establishment of the diabetes model and 1, 2, 4, and 8 weeks after the establishment. The NGF expression in dorsal horn and DRG was measured by western blot.

Results: The mechanical pain threshold decreased one week after the establishment of the diabetes model, which continued for 8 weeks. The NGF expression in the dorsal horn was reduced 2 weeks after diabetes induction and the decreased NGF expression continued for 4 weeks. However, the NGF expression in DRG was reduced one week after diabetes induction and remained at a low level for 8 weeks. Hyperalgesia occurred when the NGF expression in the DRG decreased and further reduction in the NGF expression in the dorsal horn caused concomitant allodynia. The mechanical pain threshold was significantly elevated 2 weeks after mNGF treatment.

Limitations: The course of diabetes should be much longer and there is not a precise analysis of the quantitative relation between the NGF expression in the dorsal horn/DRG and hyperalgesia/allodynia.

Conclusion: In diabetic neuropathic pain, the dynamic changes of the NGF expression in dorsal horn and DRG is involved in the development of hyperalgesia and allodynia respectively. Exogenous mNGF may relieve diabetic neuropathic pain by increasing the NGF expression in dorsal horn and DRG.

Key words: Nerve growth factor, diabetic peripheral neuropathy, pain, hyperalgesia, allodynia, dorsal horn, dorsal root ganglion

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Diabetic peripheral neuropathy (DPN) is the most common complication of diabetes and often occurs in about 15–25% of type-1 diabetic patients, and 30–40% in type-2 diabetic patients (1). Furthermore, approximately 60–90% of patients with DPN have self-reported symptoms which are characterized by burning-like pain and numbness on the skin of distal extremities, especially the lower limbs, referring to painful diabetic neuropathy (PDN) (2). Presently, the occurrence and development of PDN are poorly understood, resulting in a great amount of difficulties in clinical treatments of PDN.

It has been reported that the reduction in neurotrophic factors, such as nerve growth factor (NGF), neurotrophin-3 (NT-3), insulin-like growth factor (IGF), and vascular endothelial growth factor (VEGF) may worsen DPN (3-5). NGF is a soluble protein which is compounded by a variety of cells in the nervous, endocrine, and immune systems. NGF is an indispensable factor that maintains the growth, development, and function of sympathetic and sensory neurons. It stimulates the axon growth, maintains the axon size, prevents the post-injury death of mature neurons, and regulates various functions of the nervous system including synaptic plasticity and neurotransmission. Numerous studies have demonstrated that mast cells, macrophages, lymphocytes, fibroblasts, keratinocytes, and so on will release NGF when tissue injury and inflammation occurs, and that NGF acts as an algogenic mediator in the inflammation and neuropathic pain (6). However, the role of NGF in the neuropathic pain of diabetic patients has a unique profile. NGF expression was down-regulated in diabetic patients and exogenous NGF might improve the neuropathic pain (7). On the other hand, a fully human monoclonal antibody against NGF could be effective for treatment of DPN pain (8). Although plenty of studies are conducted on the peripheral nervous system, little is known about the relationship between the dynamic expression of NGF in dorsal horn and dorsal root ganglion (DRG) at the early stage of diabetes and the neuropathic pain.

In the present study, our results may be beneficial for understanding the dynamic changes in the NGF in the peripheral and central nervous system of patients with DPN and for interpreting the potential relationship between NGF and the hyperalgesia or allodynia.

METHODS

Establishment of the Diabetes Model

Healthy male Sprague-Dawley rats, weighing 180–200 grams, were purchased from Vital River Co., Ltd and housed in the Experimental Animal Center (specific pathogen free) for 3–5 days. The room was well-ventilated and equipped with an air filtration system. The temperature was maintained at about 23°C and humidity was at about 55%. The rats were given ad libitum access to food and water. All experimental procedures and protocols used in the present study were reviewed and approved by the Animal Care and Use Committee. The rats were fasted for 12 hours and then received streptozocin (STZ, 55 mg/kg; Sigma, USA) intraperitoneally one time. Rats in the control group were intraperitoneally administered with an equivalent volume of citric acid/sodium citrate buffer. Forty-eight hours after STZ administration, the blood glucose level was determined with a glucose analyzer. The diabetic rats were defined as those with a fasting blood glucose level greater than 16.7 mol/l; a total of 70 diabetic rats were obtained.

Experimental Grouping

Part 1

The rats were randomly assigned into 2 groups: control group (C, n = 10) and diabetes group (DM, n = 40). The rats in the diabetes group were further divided into 4 subgroups: diabetes week 1 group (DM1, n = 10; rats were sacrificed at week 1), diabetes week 2 group (DM2, n = 10; rats were sacrificed at week 2), diabetes week 4 group (DM4, n = 10; rats were sacrificed at week 4), and diabetes week 8 group (DM8, n = 10; rats were sacrificed at week 8). Six rats in each group were used for detecting NGF expression with Western blot. Our preliminary experiment and Chen's study (9) showed that almost all normal rats had no response when von Frey hair was less than 4 g. Therefore, when mechanical pain threshold was lower than 4 g, allodynia appeared. Based on the mechanical pain threshold, rats in the DM group were subdivided into 2 groups: hyperalgesia group (Hyper group), in which 50% of mechanical withdrawal threshold was decreased but not lower than 4 g, and allodynia group (Allo group), in which 50% of mechanical withdrawal threshold was lower than 4 g.

Part 2

The rats were randomly assigned into 2 groups: control group (n = 10) and treatment group (n = 30). The rats in the treatment group were assigned into 3 subgroups: saline group (S, n = 10), low dose mNGF group (mNGF1, n = 10), and high dose mNGF group (mNGF2, n = 10). The dosage of mNGF1 group and mNGF2 group was determined according to He and Yin's researches (10). Rats in the mNGF1 group and mNGF2 group were given 4 µg/kg/d and 20 µg/kg/d mouse NGF (mNGF) intraperitoneally, 2 weeks after establishment of the diabetes model, respectively. However, those in the saline group were administrated with an equivalent volume of saline, intraperitoneally. The treatment with mNGF or saline was continued for 2 weeks. The mNGF was extracted from the submaxillary glands of mice and bought from Wuhan Hitek Co., Ltd. The volume of mNGF or saline given intraperitoneally was 5 ml/kg.

Assessment of Mechanical Pain Threshold

Mechanical pain threshold was assessed with the up-down method described by Chaplan (11). All experiments were performed at 9:00–12:00 AM in a quiet environment. In brief, the rats stayed in a transparent plexiglass container for 30 minutes for environmental adaptation. Then, these rats were placed on an elevated wire grid and the plantar surface of the paw was stimulated with von Frey hairs until the hairs bent 90°. Each test consisted of an application of 6–8 von Frey hairs, with 5 minute intervals between stimuli. Quick withdrawal or licking of the paw in response to the stimulus was considered as a positive response. The threshold was considered as the lowest force that evoked a 50% paw withdrawal.

Detection of NGF Expression by Western Blot

The rats were anesthetized with chloral hydrate (300 mg/kg), intraperitoneally. The dorsal horn and DRG of L4-6 were obtained and stored in liquid nitrogen. The protein was extracted and protein concentration was determined by BCA method. Then, 50 µg of protein were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and were transferred onto polyvinylidene fluoride membrane (300 mA, 2 h). The membrane was blocked with 5% non-fat milk for 1 hour and incubated with rabbit anti-NGF antibody (1:400; Santa Cruz, USA) overnight at 4°C. After being washed, the membrane was incubated with goat anti-rabbit IgG conjugated to the horseradish peroxidase (1:8000; Kangcheng, China) for 45 minutes. Then, signal

developing was performed with an enhanced chemiluminescent (ECL) kit in a dark room. The mean density of the gray scale bands was quantified using an image analyzer (XK-100, Shanghai, China) after the film was scanned. In order to normalize the data to account for any minor variations in protein loading, the NGF band density was normalized with the corresponding GAPDH band density.

Statistical Analysis

All statistical analyses were performed by SPSS 10.0 statistical software. The quantitative data were presented as mean ± standard deviation ($\bar{x} \pm s$). T-test was used for comparisons between 2 means, and one way ANOVA was applied for comparisons among multiple groups, followed by comparison between either 2 groups with LSD. The relationship between NGF expression in dorsal horn/DRG and mechanical pain threshold was analyzed by Spearman linear correlation analysis.

RESULTS

The General State of Experimental Rats

The body weight of the control rats increased after one week and continued to do so for 8 weeks. However, the body weight of the diabetic rats was became significantly lower than that of the control rats and did not change significantly up to 8 weeks. Compared with the control group, the plasma glucose of all diabetic rats increased up to 16.7 mmol/L and remained high after 8 weeks.

Changes of Mechanical Pain Threshold

One week after diabetes modeling, the mechanical pain threshold of diabetic rats decreased to 4.37 ± 1.10 g and hyperalgesia was observed and continued for 8 weeks. Compared with the control group, the mechanical pain threshold of diabetic rats 1, 2, 4, and 8 weeks after diabetes modeling decreased significantly ($P < 0.0001$). Two and 4 weeks after diabetes modeling, hyperalgesia and allodynia were noted. The mechanical pain threshold of diabetic rats reached a minimum (3.22 ± 2.51 g) 4 weeks after diabetes modeling (Fig. 1).

Expression of NGF in Dorsal Horn and DRG

The expression of NGF in dorsal horn and DRG was down-regulated one week after diabetes induction. The NGF expression in dorsal horn of diabetic rats 2 and 4 weeks after diabetes modeling was about 58.9% and 61.9% of the control rats, respectively ($P = 0.003$,

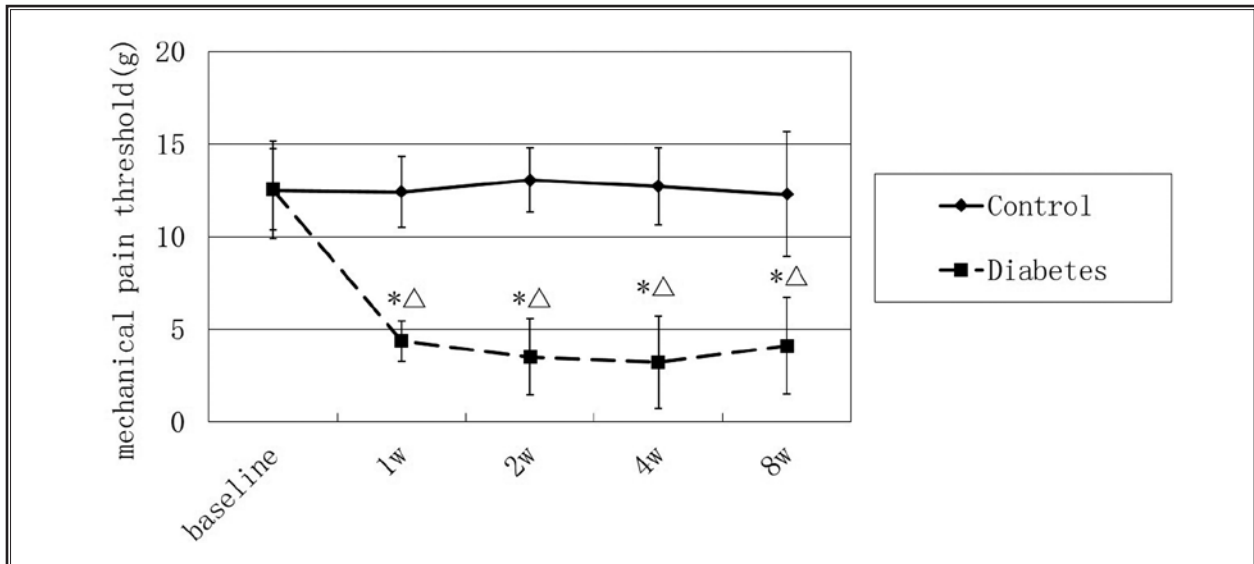


Fig. 1. Changes in the mechanical pain threshold of rat hind paws. Data are presented as mean \pm SD, n = 10.

*: Compared with control group, statistical significance.

Δ : Compared with baseline level, statistical significance.

Diabetes 1w: compared with control group, $P < 0.0001$; compared with baseline, $P < 0.0001$.

Diabetes 2w: compared with control group, $P < 0.0001$; compared with baseline, $P < 0.0001$.

Diabetes 4w: compared with control group, $P < 0.0001$; compared with baseline, $P < 0.0001$.

Diabetes 8w: compared with control group, $P < 0.0001$; compared with baseline, $P < 0.0001$.

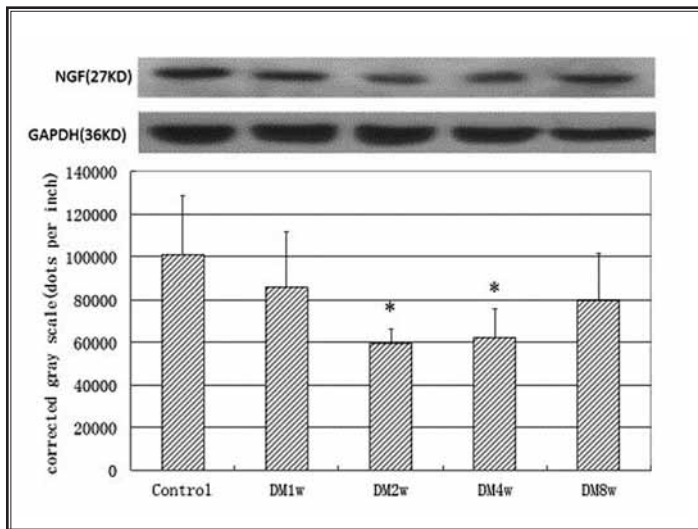


Fig. 2. Changes in the NGF expression in dorsal horn (representative bands of NGF and histogram of NGF expression). Data are presented as mean \pm SD, n = 6.

*: Compared with control group, statistical significance.

DM1w: compared with control group, $P = 0.247$.

DM2w: compared with control group, $P = 0.003$.

DM4w: compared with control group, $P = 0.006$.

DM8w: compared with control group, $P = 0.103$.

0.006). In addition, the NGF expression in DRG of diabetic rats 1, 2, 4, and 8 weeks after diabetes modeling was 54.3%, 42.0%, 43.7%, and 55.2% of the control rats, respectively ($P = 0.004, 0.001, 0.002, 0.014$, respectively). Furthermore, the NGF expression in dorsal horn and DRG of diabetic rats reached a minimum 2 weeks after diabetes modeling (Fig. 2-3).

Relationship Between NGF Expression and Mechanical Pain Threshold

Spearman linear correlation analysis was applied to analyze the relationship between the NGF expression and mechanical pain threshold determined before being sacrificed. The results showed the NGF expression in dorsal horn and DRG was positively related with the mechanical pain threshold ($r = 0.460, P = 0.002$; $r = 0.557, P = 0.0001$, respectively) (Fig. 4-5). In addition, the mechanical pain thresholds of diabetic rats of Hyper group (n = 6) and Allo group (n = 18), in which NGF expression would be detected with Western blot, were 4.62 ± 0.43 g and 2.52 ± 0.96 g, respectively.

Then, the NGF expression was compared between Hyper group and Allo group. The result indicated the ratio of NGF expression in dorsal horn of Hyper group to that of Allo group was 1.50 ($P = 0.002$) and that in DRG was 1.17 ($P = 0.065$) (Fig. 6).

Effects of mNGF on the Mechanical Pain Threshold

No significant changes in the mechanical pain threshold of the control group and saline group were observed after mNGF treatment. Two weeks after mNGF treatment, the mechanical pain threshold of mNGF1 group and mNGF2 group increased to 8.05 ± 2.88 g and 6.28 ± 3.42 g, respectively, which did not reach the baseline threshold. Significant difference in the mechanical pain threshold was observed between before and after mNGF treatment in mNGF1/mNGF2 groups ($P = 0.0002, 0.0005$), as well as between mNGF1/mNGF2 groups and saline group or control group ($P = 0.0002, 0.0004, 0.016, P < 0.0001$, respectively). However, there was no significant difference between mNGF1 group and mNGF2 group ($P = 0.202$) (Fig. 7).

Effects of mNGF on the Expression of NGF in the Dorsal Horn and DRG

Compared with the control group, the NGF expression in dorsal horn and DRG of the saline group decreased dramatically before and after mNGF treatment ($P = 0.006, 0.002$). After mNGF treatment, the NGF expression in dorsal horn of mNGF1 group was not up-regulated markedly (compared with saline group, $P = 0.545$; compared with control group, $P = 0.017$). But, that of mNGF2 group was increased (compared with saline group, $P = 0.045$; compared with control group, $P = 0.137$; compared with mNGF1 group, $P = 0.282$) (Fig. 8). After mNGF treatment, the NGF expression in DRG of mNGF1 group was up-regulated (compared with saline group, $P = 0.039$; compared with control group, $P = 0.002$). In addition, that of mNGF2 group was also increased (compared with saline group, $P = 0.029$; compared with control group, $P = 0.615$; compared with mNGF1 group, $P = 0.033$) (Fig. 9).

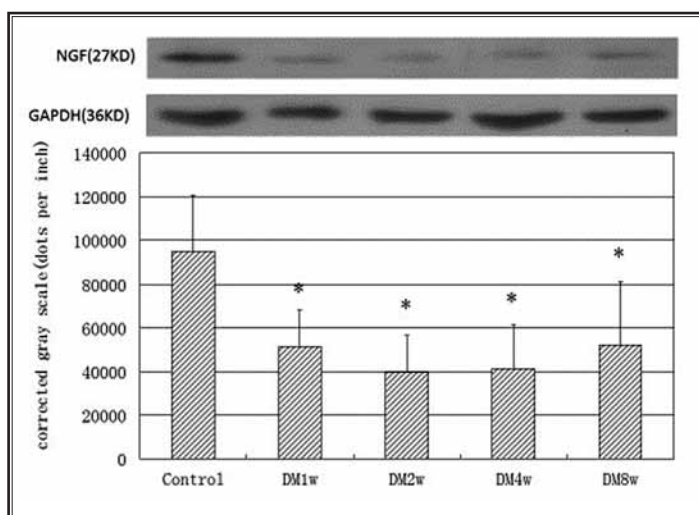


Fig. 3. Changes in the NGF expression in DRG (representative bands of NGF and histogram of NGF expression). Data are presented as mean \pm SD, $n = 10$.

*: Compared with control group, statistical significance DM1w: compared with control group, $P = 0.004$.
DM2w: compared with control group, $P = 0.001$.
DM4w: compared with control group, $P = 0.002$.
DM8w: compared with control group, $P = 0.014$.

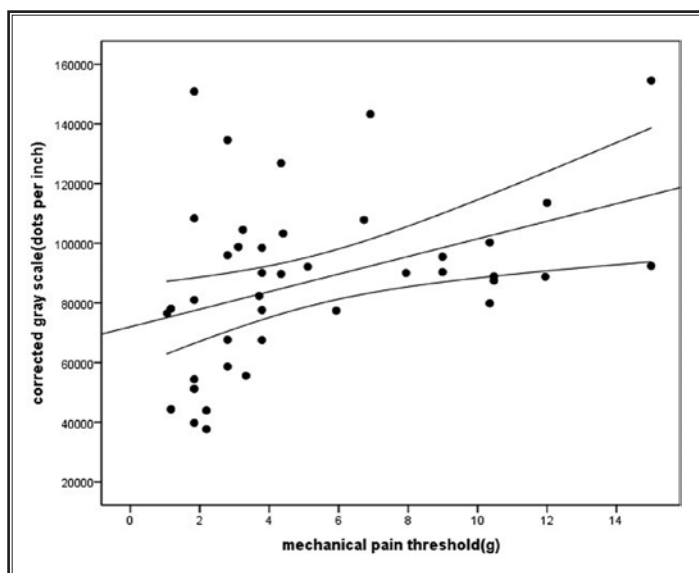


Fig. 4. The relationship between the NGF expression in dorsal horn and mechanical pain threshold.

The NGF expression was positively related with the mechanical pain threshold ($r = 0.460, P = 0.002$).

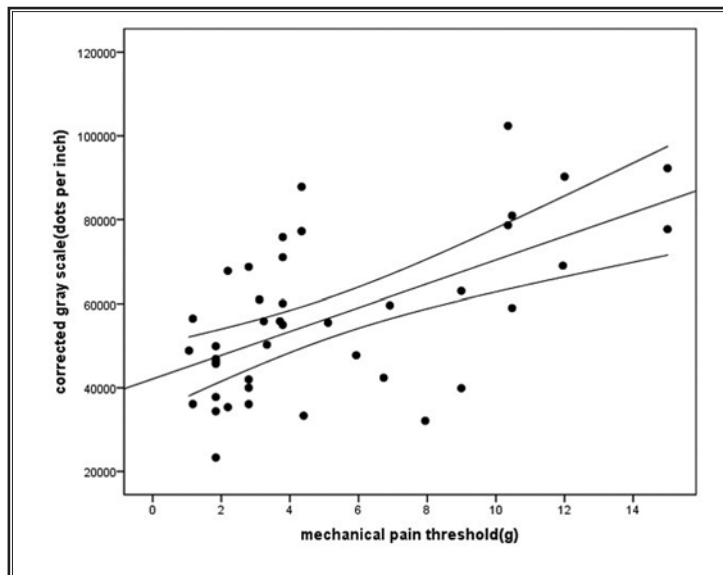


Fig. 5. The relationship between the NGF expression in DRG and mechanical pain threshold.

The NGF expression was positively related with the mechanical pain threshold ($r = 0.557$, $P = 0.0001$).

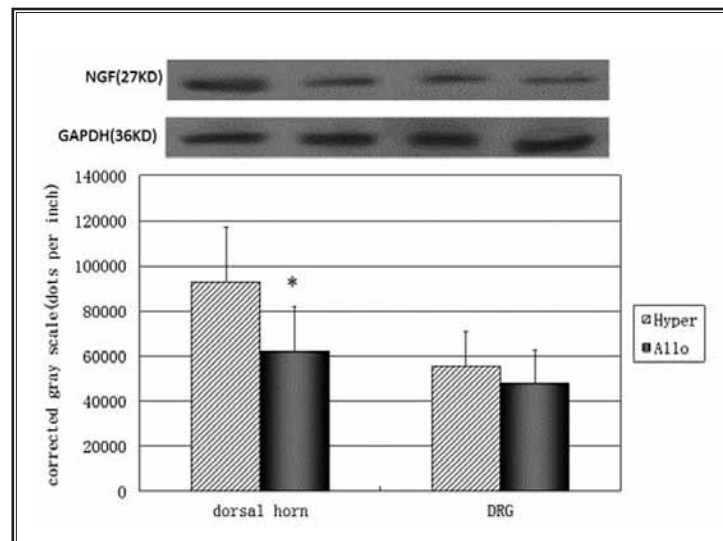


Fig. 6. The NGF expression in dorsal horn and DRG of Hyper group and Allo group.

*: Compared with Hyper group, statistical significance.

In dorsal horn: Allo group, compared with Hyper group, $P = 0.002$.

In DRG: Allo group, compared with Hyper group, $P = 0.065$.

DISCUSSION

Numerous studies have indicated the pain threshold for diabetic rats, For example, the heat and mechanical pain threshold would decrease after 2 weeks, while chemical stimulus pain threshold would decrease in 4 weeks (12). Mechanical pain sensitivity would appear within 8 days after STZ injection and would continue for at least 4 weeks, and elucidated that mechanical pain sensitivity in diabetic rats would appear within 8 days after STZ injection and would continue for at least 4 weeks (13). However heat pain threshold had not been changed and appeared to be even higher (14). The reason that various kinds of nociceptive pain induce different changes in pain threshold may be correlated to different types of nerve fibers ($A\alpha$, $A\delta$, C, etc.) and neurotransmitters (substance P, neurokinin, glutamic acid, etc.). These nerve fibers and neurotransmitters participate in transmitting nociceptive pain from peripheral nociceptors to the central nervous system (13). Another study also showed that the mechanical allodynia was mainly conducted by myelinated fibers, while thermal hurtful experience was mainly transmitted through C fiber (15).

The pathogenesis of diabetic neuropathy is still not completely understood, and is currently considered to be a result of various factors including metabolic factors, vascular factors, oxidative stress, abnormalities in neurotrophic factors, immune factors, genetic factors, and so on. Recently, effects of neurotrophic factors on diabetic neuropathy have become a research hotspot. For example, changes of NGF, NT-3, and IGF seem to worsen diabetic neuropathy, while VEGF may attenuate pain behavior and prevent neuronal stress through an effect on transient receptor potential ankyrin 1 (TRPA1) activity in a model of type I diabetes. Among neurotrophic factors, NGF is considered to be the most important one for diabetic neuropathy (3-5).

Progression has been made in the relationship between the NGF and diabetic neuropathy. NGF expression in the vagus nerve of rats with STZ induced diabetes

decreased (16). mRNA expression of NGF in the calf muscle and sciatic nerve of rats markedly decreased at the early stage of diabetes (17). In addition, studies also reported the levels of NGF and SP in the skins of patients with diabetic neuropathy were significantly decreased and were correlated with the severity of sensory impairment (18,19). These findings might be explained by the insulin deficiency and high level of blood glucose decreased by the expression of NGF in the target tissues, accompanied by reduced synthesis of NGF in the Schwann cells. Moreover, the decreased affinity of NGF receptor (TrkA) and impaired reverse axoplasmic transport may be potential causes of down-regulation of NGF expression (20,21). The normal expression of NGF is indispensable for maintaining the normal functions of sensory and sympathetic nerves. NGF is implicated in the morphological diversity and promotion of nerve regeneration and neurotransmitter expression (22). It not only inhibits neural degeneration resulting in delayed disease progression, but stimulates axon growth and neurogenesis. In addition, NGF is also involved in the angiogenesis and islet development. In diabetic neuropathy, the functions of NGF are impaired and the expression of NGF related genes are also altered. The synthesis of neurofilament, microtubule, and neurotransmitter is decreased, leading to neuroaxonal dystrophy and impaired nerve regeneration or even neuron atrophy, shedding, and missing. These changes are important factors involved in the occurrence and development of diabetic neuropathy. The efficacy of electro acupuncture for diabetic neuropathy might depend on its actions on spinal/peripheral NGF synthesis/utilization and normalization of the levels of several sensory neuromodulators (23). Although the down-regulation of NGF expression in diabetes has been confirmed in numerous studies, no study has reported the dynamic changes in the NGF expression in the peripheral and central nervous system (dorsal horn and DRG) at the early stage of diabetes, which was investigated in the present study. In addition, the relationship between the NGF expression

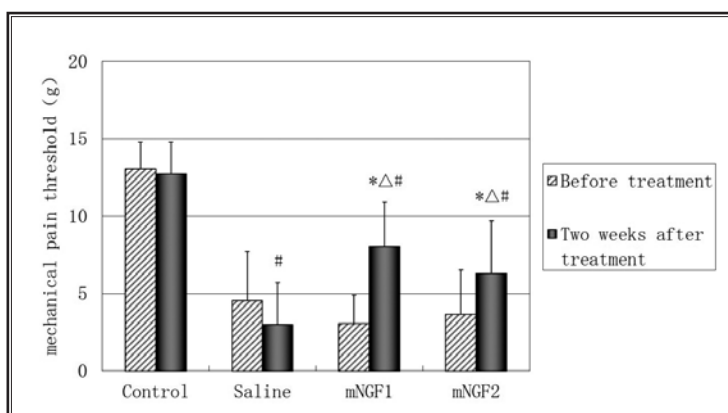


Fig. 7. Changes in the mechanical pain threshold before and after mNGF treatment. Data are presented as mean \pm SD, $n = 10$.

*: Compared with before mNGF treatment, statistical significance.
 Δ : Compared with Saline group, statistical significance.
 $\#$: Compared with control group, statistical significance.
 Saline group: compared with control group, $P < 0.0001$.
 mNGF1 group: compared with before mNGF treatment, $P = 0.0002$; compared with Saline group, $P = 0.0002$; compared with control group, $P = 0.0004$.
 mNGF2 group: compared with before mNGF treatment, $P = 0.0005$; compared with Saline group, $P = 0.016$; compared with control group, $P < 0.0001$; compared with mNGF1 group, $P = 0.202$.

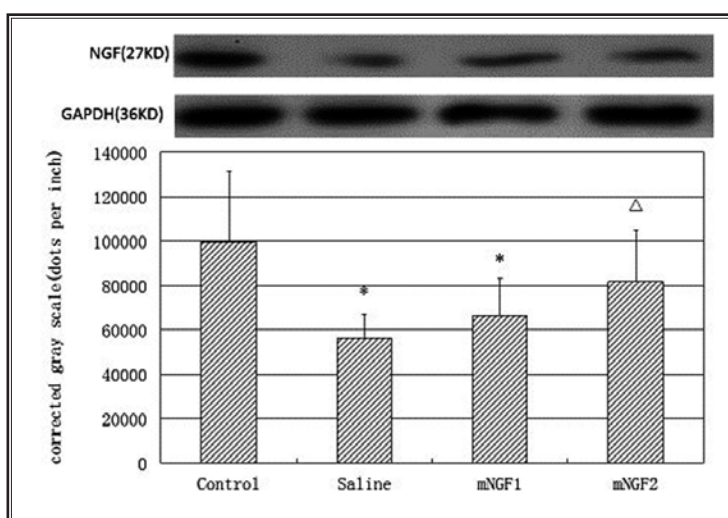


Fig. 8. Changes in the NGF expression in dorsal horn after mNGF treatment (representative bands of NGF and histogram of NGF expression). Data are presented as mean \pm SD, $n = 10$.

*: Compared with control group, statistical significance.
 Δ : Compared with Saline group, statistical significance.
 Saline group: compared with control group, $P = 0.006$.
 mNGF1 group: compared with Saline group, $P = 0.545$; compared with control group, $P = 0.017$.
 mNGF2 group: compared with Saline group, $P = 0.045$; compared with control group, $P = 0.137$; compared with mNGF1 group, $P = 0.282$.

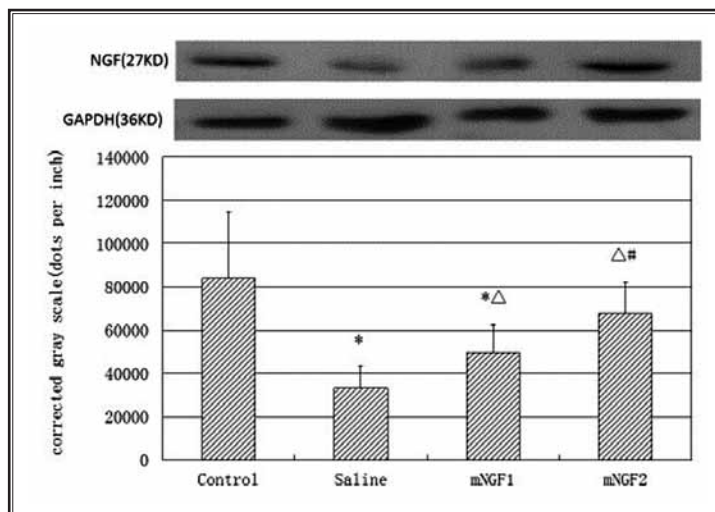


Fig. 9. Changes in the NGF expression in DRG after mNGF treatment (representative bands of NGF and histogram of NGF expression).

*: Compared with control group, statistical significance.

Δ: Compared with Saline group, statistical significance.

#: mNGF2 group vs. mNGF1 group, statistical significance.

Saline group: compared with control group, $P = 0.002$.

mNGF1 group: compared with Saline group, $P = 0.039$; compared with control group, $P = 0.002$.

mNGF2 group: compared with Saline group, $P = 0.029$; compared with control group, $P = 0.615$; compared with mNGF1 group, $P = 0.033$.

in dorsal horn and DRG and the mechanical pain threshold was also explored. This study may be helpful for understanding the role of NGF in the PDN and for early intervention.

Our results indicated that the NGF expression in both dorsal horn and DRG decreased. However, the decreased NGF expression in DRG occurred one week after diabetes induction, while that in dorsal horn occurred 2 weeks after diabetes induction. Additionally, the decreased NGF expression in DRG continued for 8 weeks and the NGF expression in dorsal horn almost reached the baseline level 8 weeks after diabetes induction. The NGF expression in DRG and dorsal horn of diabetic rats was 42% and 59% of control rats, respectively. The extent of decreased NGF expression in DRG was higher than that in dorsal horn. These findings implied that the reduced NGF expression initially occurred in the peripheral nervous system. It can be explained that a high level of blood glucose in diabetic patients will decrease the expression of NGF in some target cells including glial cells, fibroblasts, Schwann cells, etc. In addition, the axonal retrograde transport is impaired and the expression of NGF in the soma of peripheral neurons is decreased firstly.

Few studies reported the NGF expression in spinal cord. Our results showed the NGF expression in dorsal horn decreased. This might be a result of decreased NGF expression in the peripheral

nervous system, which decreases the amount of NGF by axonal retrograde transport. Therefore, the time and extent of decreased NGF expression were different between dorsal horn and DRG. Though it may be temporary, the NGF expression almost reached the baseline level and had a rising trend in dorsal horn and DRG respectively, which could be explained by the compensatory expression of NGF. For example, the expression of tumor necrosis factor alpha (TNF- α) increases in rats with diabetes (24), and TNF- α can promote the release of NGF in astrocytes (25). In addition, the hyperalgesia was observed 1, 2, 4, and 8 weeks after diabetes induction, consistent with the time of NGF decrease in DRG. Allodynia was noted at 2 and 4 weeks after diabetes induction, consistent with the time of NGF decrease in dorsal horn. Moreover, the NGF expression in rats with allodynia was markedly lower than in those with hyperalgesia. These findings suggested that hyperalgesia might be related with decreased NGF expression in DRG and allodynia with the reduced expression of NGF in dorsal horn.

A variety of measures have been taken to treat diabetic neuropathy, but an effective strategy has yet to be made available. Proper blood glucose control is still the basis in the treatment of diabetic neuropathy. Because the endogenous NGF production is reduced under diabetic conditions, exogenous NGF supplement may be beneficial for the improvement of PDN and will become a favorable strategy for the diabetic patients with neuropathy. The augmentation of NGF protein in diabetic animals established by STZ would improve the decreased pain and thermal sense and the abnormal conduction of sensory nerve fiber (26). rhNGF was the best candidate to advance in diabetic neuropathy (27). Moreover, a clinical trial has also indicated the therapeutic effects of rhNGF on neurological diseases, and they are being recognized (28). On the contrary, other studies had contradictory results demonstrating that no profound effects of rhNGF on diabetic neuropathy (29). Therefore, the role of rhNGF in the diabetic neuropathy is required to be further studied. mNGF had

been shown to be clinically effective for the treatment of peripheral nerve injury (30) and chronic peripheral polyneuropathy (31). Meanwhile, mNGF also had positive effects on nerve impairment in rats (10). Therefore, we choose mNGF, the first isolation of NGF, which was extracted from the submaxillary glands of mice (32), as the intervention to diabetic rats in this study. There was no study thus far that reported the effects of early administration of mNGF on the mechanical pain threshold of diabetic rats. In the present study, a low dose high dose of mNGF were administered for 2 weeks and the NGF expression in dorsal horn and DRG increased to different extents, accompanied by the restoration of mechanical pain threshold. As previously mentioned, reduced synthesis of NGF and impaired reverse axoplasmic transport may be potential causes of PDN and the down-regulation of NGF expression in dorsal horn and DRG. We knew that exogenous NGF could increase the NGF expression in the target tissues (7) and repair the damaged axonal retrograde transport through the activation of PI3/Akt and MAPK/ERK signal pathway (33). Therefore, in the present study, the NGF expression in dorsal horn and DRG increased to different levels after the treatment of exogenous mNGF. The increased NGF expression may be beneficial for the axon growth and regeneration, and also for the survival of neurons and tissue repair. Additionally, the ratio of NGF expression in DRG of mNGF1 group and mNGF2 group to that of saline group was 1.47 and 2.04, respectively. The ratio of NGF expression in dorsal horn of mNGF1 group and mNGF2 group to that of saline group was 1.18 and 1.45, respectively. These results indicated more profound improvement in the peripheral nerves than in the central nerves after mNGF treatment. These results also confirmed our hypothesis that the decreased NGF expression at the early stage of diabetes occurred in the peripheral nerves. Of note, allodynia was not observed in more than 80% of diabetic rats with allodynia after mNGF treatment and switched into hyperalgesia. However, the hyperalgesia in the diabetic rats was only relieved and not completely abolished. These findings suggested the PDN was a result of interaction between multiple factors. Furthermore, the NGF expression may play a more important role in the pathogenesis of al-

lodynia than that of hyperalgesia.

Another important finding was that the increased NGF expression in dorsal horn and DRG after mNGF treatment was in a dose-dependent manner. In the mNGF2 group, the NGF expression reached the baseline level, but the mechanical pain threshold was not proportionally increased. The mechanical pain threshold was still lower than that of the control group and had a tendency to decrease compared to the mNGF1 group. These findings suggested the double-sword effects of NGF. It has been shown that increased NGF expression could stimulate the expression of P substance (34), vanilloid type 1 receptors (VR1) (35), and acid-sensing ion channel 3 (36); these factors are involved in the transmission of pain signals. In addition, the release of histamine, 5-HT, prostaglandin, and bradykinin in the peripheral inflammation sites were also increased by NGF, which elevated the sensitivity of sensors and induced sprouting of A β fibers (37). Long-term mechanical hyperalgesia and thermal hyperalgesia could be induced after local or systemic administration of NGF (38,39), and hyperalgesia of the sciatic nerve also occurred after NGF treatment (40). These studies may explain why higher doses of mNGF have less effect on mechanical pain threshold than smaller doses.

There were some limitations in this study. For example, there was no data about mechanical pain threshold and NGF expression 8 weeks after establishment of the diabetes model, and the long-term dynamic change of mechanical pain threshold and NGF expression need further investigation.

CONCLUSION

Despite its limitations, this study clearly indicates that the dynamic changes in the NGF expression in the peripheral and central nervous system, at the early stage of diabetes, were involved in the pathogenesis of PDN. Hyperalgesia will occur when the NGF expression in DRG is decreased, and further reduction in the NGF expression in dorsal horn will cause concomitant allodynia. Additionally, exogenous mNGF may relieve PDN through increasing the NGF expression in dorsal horn and DRG.

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