

Nerve growth factor enhances the therapeutic effect of mesenchymal stem cells on diabetic periodontitis

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Abstract. Patients with diabetes frequently suffer from periodontitis, which progresses rapidly and is difficult to cure. Mesenchymal stem cell (MSC) transplantation may effectively treat periodontitis, but high glucose limits its therapeutic effect in diabetes. Nerve growth factor (NGF) has the functions of cell protection, anti-apoptosis and immune regulation, and may have potential application in diabetic periodontitis. In the present study, flow cytometry indicated that NGF inhibited MSC apoptosis induced by high glucose. Of note, high glucose promoted the transformation of MSCs into the proinflammatory type. NGF inhibited this transformation of MSCs under diabetic conditions and further decreased the proportion of T cells and monocytes/macrophages among lymphocytes. An animal model of diabetic periodontitis was constructed and MSC transplantation was demonstrated to reduce alveolar bone loss caused by diabetes. NGF enhanced the therapeutic effect of MSCs and maintained transplanted MSC survival in periodontal tissue of diabetic mice. Immunohistochemical analysis of periodontal tissues suggested that in the NGF group, infiltration of T cells and macrophages was reduced. Neurotrophic receptor tyrosine kinase 1 was indicated to have a key role in these effects of NGF. In conclusion, NGF may enhance the therapeutic effect of MSCs on diabetic periodontitis by protecting the cells and promoting the transformation of MSCs into the immunosuppressive type.

Introduction

Periodontitis is a destructive disease with periodontal tissue inflammation, which seriously affects oral health. In addition to plaque and calculus, certain systemic diseases are also important contributors to periodontitis (1). Among them, the

association between diabetes and periodontitis has attracted the attention of researchers (2). Clinical studies have indicated that the prevalence of periodontitis in diabetic patients was significantly higher than that in non-diabetic patients. The immune dysfunction and the decline of the host's defense ability are important causes of periodontitis in diabetes (3). Furthermore, diabetes may also increase apoptosis of osteoblasts and periodontal fibroblasts, further aggravating the development of periodontitis. Periodontitis is also a risk factor for diabetes (4). If periodontitis is not effectively controlled, the bacteria in periodontal pockets and the toxins they secrete trigger a long-term chronic inflammatory response, damaging the function of islet β -cells and increasing the risk of diabetes (5).

Mesenchymal stem cells (MSCs) may be isolated from bone marrow, fat and umbilical cord. There are numerous immunosuppressive molecules expressed on the MSCs, which have certain immune regulation functions (6). MSCs also secrete numerous cytokines to optimize the local microenvironment and promote tissue repair via paracrine signaling. At present, MSCs are widely used in the treatment of diabetes and its complications with good therapeutic effects (7,8). MSC therapy for diabetic foot has entered clinical practice, with results indicating that MSCs may promote healing of diabetic ulcers and repair of nerves (9,10). For periodontitis, MSCs are able to repair bone defects and reduce inflammatory response (11). However, in diabetes, MSC function is seriously damaged and their apoptosis rate is significantly increased, which affects the therapeutic effect of MSCs in periodontitis (12). Numerous factors have an important role in high glucose (HG)-induced damage, including reactive oxygen species and mitochondrial dysfunction. In addition, hyperosmosis may cause apoptosis and impaired function of cells. Therefore, protecting MSCs against HG-induced damage is crucial in the treatment of diabetic periodontitis.

Nerve growth factor (NGF) is an important nerve factor that maintains nerve growth, differentiation and axon production. NGF may reduce cerebral ischemia-reperfusion injury and promote neuronal repair (13). Numerous studies have demonstrated that NGF also has tissue and cell protective functions, reducing apoptosis caused by ischemia and inflammation (14). The effect of NGF on MSCs has been widely reported. NGF may induce MSC differentiation, inhibit MSC apoptosis and enhance MSC paracrine signaling (15-17). However, the effect of NGF on immune regulation of MSCs has remained elusive.

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In the present study, the effect of NGF on the therapeutic efficacy of MSCs in diabetic periodontitis was assessed and the underlying mechanisms were investigated, so as to provide a novel agent for clinical treatment.

Materials and methods

Cell culture and grouping. MSCs used in the present study were human bone marrow MSCs (hMSCs) purchased from Guangzhou Saiye Biotechnology Co. hMSCs were cultured in DMEM/F12 medium (Hyclone; Cytiva) containing 10% fetal bovine serum (Hyclone; Cytiva). MSCs of the 3rd–5th generation were used for cell and animal experiments. For the *in vitro* experiments, cells were divided into four groups and accordingly cultured in medium containing the following: Low-glucose (LG) group (5.6 mM glucose), LG+NGF group (5.6 mM glucose and 10 ng/ml NGF), HG group (30 mM glucose), HG+NGF group (30 mM glucose and 10 ng/ml NGF) and neurotrophic receptor tyrosine kinase 1 (TrkA) inhibition group (30 mM glucose, 10 ng/ml NGF and TrkA inhibitor GW441756). After 48 h of stimulation, the cell supernatants from each group were collected and the concentrations of TGF- β (cat. no. EHJ-10099), IL-10 (cat. no. EHJ-10479), TNF- α (cat. no. EHJ-10039) and IL-6 (cat. no. EHJ-10292) in the supernatants were detected by ELISA (all purchased from Xiamen Huijia Biotechnology Co., Ltd.).

Apoptosis experiment. MSCs were digested with 0.25% trypsin (Hyclone; Cytiva) and seeded in 6-well plates (Corning, Inc.) at a density of 2×10^4 cells/well. The treatment of MSCs was as aforementioned. After 48 h of 10 ng/ml NGF stimulation, MSCs were digested with 0.25% trypsin and suspended in 100 μ l PBS. Annexin V-FITC and PI (Roche Diagnostics) were added, followed by incubation at room temperature in the dark for 15 min. Binding buffer (400 μ l) was added and the ratio of apoptotic cells was detected by flow cytometry (BD LSRFortessa X-20; BD Biosciences). The proportion of apoptotic cells was quantified using FlowJo software (version 7.6; BD Biosciences).

MTT assay. MSCs were digested with 0.25% trypsin and seeded in 96-well plates at a density of 6×10^3 cells/well. MSCs were treated as aforementioned. After 48 h of 10 ng/ml NGF stimulation, 20 μ l MTT solution (5 mg/ml) was added to each well and the culture was continued for 4 h. After discarding the supernatant, 150 μ l DMSO (Sigma-Aldrich; Merck KGaA) was added to each well. After 10 min of low-speed oscillation, the absorbance value of each well at 490 nm was measured with an ELISA plate reader.

Flow cytometry. After 48 h of NGF stimulation, MSCs were digested with 0.25% trypsin and suspended in 100 μ l PBS. Toll-like receptor (TLR)4-PE (cat. no. 564215), TLR3-APC (cat. no. 565984), human leukocyte antigen G (HLA-G)-FITC (cat. no. 751657) (all purchased from BD Biosciences) were added and used as provided by the manufacturer, followed by incubation at room temperature in the dark for 15 min. After washing with PBS three times, the mean fluorescence intensity of the fluorophores for TLR4, TLR3 and HLA-G was detected by flow cytometry (BD LSRFortessa X-20; BD Biosciences).

Proportion of apoptotic cells was quantified using FlowJo software (version 7.6; BD Biosciences).

Co-culture experiment. After 48 h of NGF stimulation, the supernatant was discarded and fresh medium was added after washing the cells with PBS for three times. Male, eight-week-old C57BL/6 mice (Charles River Laboratories, Inc.) were raised in a suitable environment (temperature, 22°C; relative humidity, 50%; access to food and water *ad libitum*). A total of six mice (20 g; kept under a 12:12 h light and dark cycle) were anesthetized (400 mg/kg chloral hydrate, intraperitoneal injection). A 1-ml needle was used to pierce the heart and collect blood from the heart in a terminal procedure. According to the manufacturer's protocol, lymphocytes were isolated with lymphocyte separation fluid (Tianjin TBD Biotechnology Co., Ltd.) from the mouse blood collected, and then added to MSC medium. According to the treatment of MSCs, the experiment was divided into five groups: LG-MSC group (1×10^5 lymphocytes and 1×10^4 MSCs were co-cultured in DMEM/F12 medium containing 5.6 mM glucose), LG+NGF-MSC group (1×10^5 lymphocytes and 1×10^4 MSCs were co-cultured in DMEM/F12 medium containing 5.6 mM glucose and 10 ng/ml NGF), HG-MSC group (1×10^5 lymphocytes and 1×10^4 MSCs were co-cultured in DMEM/F12 medium containing 30 mM glucose), HG+NGF-MSC group (1×10^5 lymphocytes and 1×10^4 MSCs were co-cultured in DMEM/F12 medium containing 30 mM glucose and 10 ng/ml NGF) and TrkA-inhibited MSC group (1×10^5 lymphocytes and 1×10^4 MSCs were co-cultured in DMEM/F12 medium containing 30 mM glucose, 10 ng/ml NGF and 10 ng/ml GW441756). After 48 h of incubation, CD45-PE (cat. no. 560975), CD3-APC (cat. no. 555335) and CD14-FITC (cat. no. 557153) (all purchased from BD Biosciences) were added and used as provided by the manufacturer, followed by incubation at room temperature in the dark for 15 min. After washing with PBS three times, the ratio of CD45⁺CD3⁺T cells and CD14⁺ monocytes/macrophages was detected by flow cytometry (BD LSRFortessa X-20; BD Biosciences). Proportion of apoptotic cells was quantified using FlowJo software (version 7.6; BD Biosciences). The use of mice in this experiment was approved by the Ethics Committee of The Air Force Characteristic Medical Center (Beijing, China).

Animal experiment. All experiments were approved by the Ethics Committee of The Air Force Characteristic Medical Center (Beijing, China) and were performed in accordance with the institutional regulations. Male, eight-week-old C57BL/6 mice (Charles River) were raised in a suitable environment (temperature, 22°C; relative humidity, 50%; access to food and water *ad libitum*). C57BL/6J mice were intraperitoneally injected with 40 mg/kg streptozotocin once a day for 5 days. Fasting blood glucose levels of >16.7 mmol/l determined in three consecutive measurements (interval of 1 day) were considered to indicate that the diabetic mouse model was successfully constructed and the success rate of establishing the model was 72.73%. After 1 week of feeding on normal food, the periodontitis model started to be established, the brief process is as follows. Mice were first anesthetized with chloral hydrate

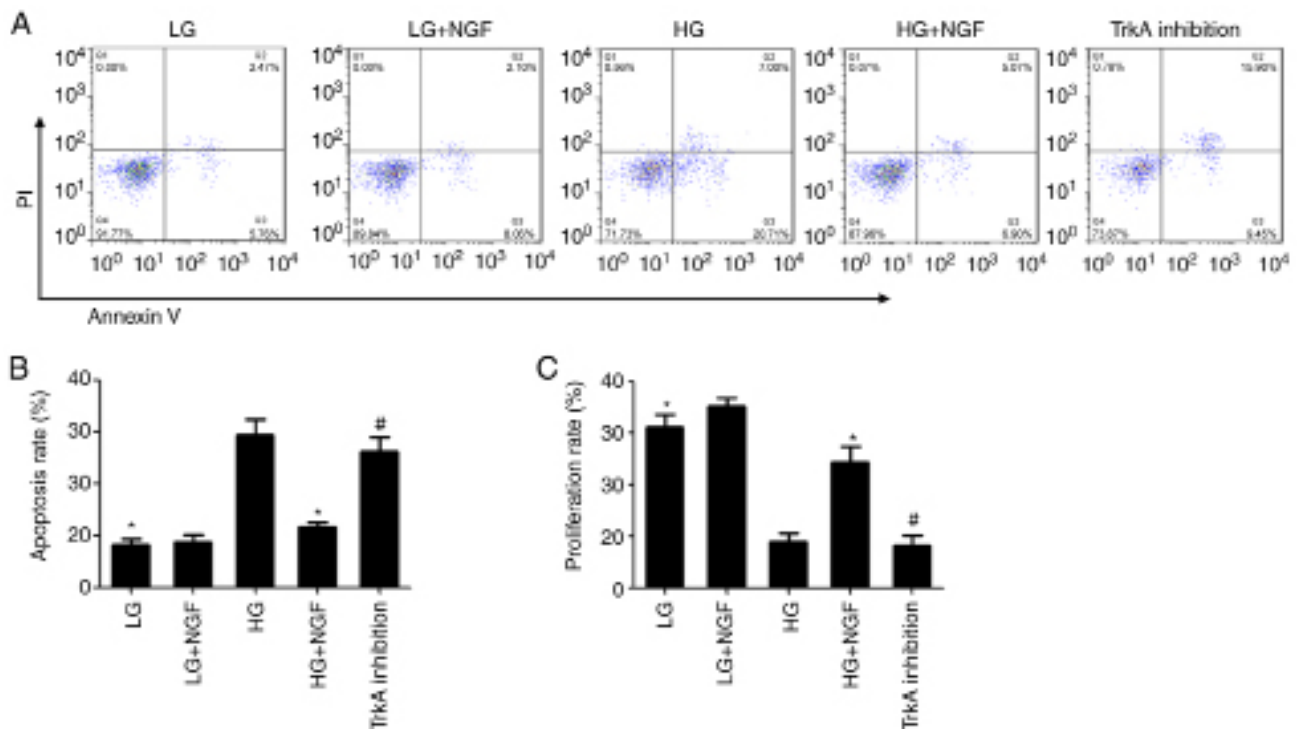


Figure 1. NGF inhibits MSC apoptosis induced by HG. (A) Flow cytometry was used to detect MSC apoptosis. (B) Compared with that in the HG group, the apoptotic rate of MSCs in the HG+NGF group decreased by 60.98%. TrkA inhibition was able to completely block this effect of NGF. (C) The proliferation rate of the HG+NGF group was 24.5% and the difference compared with the HG group was statistically significant. TrkA inhibition completely blocked this effect of NGF. Values are expressed as the mean \pm standard deviation (n=6). *P<0.05 vs. HG; #P<0.05 vs. HG+NGF. TrkA, neurotrophic receptor tyrosine kinase 1; Q, quadrant; MSC, mesenchymal stem cell; NGF, nerve growth factor; HG, high glucose; LG, low glucose.

(400 mg/kg, intraperitoneal injection). Subsequently, silk thread (5-mm diameter) was slid into the proximal and distal adjacent spaces of the maxillary second molars on both sides of the mouse, and the knots were tied on the buccal side and pressed against the gingival groove. After 4 weeks of ligation, the ligated threads were carefully removed. The mice were divided into four experimental groups: Model group (PBS was injected through the tail vein, n=10), MSC group (1×10^6 hMSCs were injected through the tail vein, n=10), NGF group (1×10^6 NGF pre-treated hMSCs were injected through the tail vein, n=10) and TrkA inhibition group (1×10^6 NGF and GW441756 pre-treated hMSCs were injected through the tail vein, n=10). All injections were performed once in each animal. The fur color, diet and behavior of the mice were monitored daily. After two weeks, mice were euthanized by anesthesia (400 mg/kg chloral hydrate, intraperitoneal injection) followed by cervical dislocation, and the alveolar bones and periodontal tissues were collected. After fixing with 4% paraformaldehyde, paraffin sections (4- μ m thick) were prepared. Certain sections were stained with H&E to detect basic pathological changes. Immunohistochemistry was performed in other sections to detect the number of anti-human nuclear antibody (ANA)-positive transplanted hMSCs (18), CD3⁺T cells and CD68⁺ macrophages (ANA cat. no. ab215396; CD3 cat. no. ab16669; CD68 cat. no. ab125212; all purchased from Abcam) and the antibodies were used as provided by the manufacturer. Transplanted hMSCs, CD3⁺T cells and CD68⁺ macrophages were directly counted under a light microscope by eye (Eclipse E200; Nikon Corporation).

Statistical analysis. Statistical analyses were performed using SPSS 19.0 (IBM Corp.). Values are expressed as the mean \pm standard deviation. Data comparisons were performed by ANOVA and Bonferroni's post-hoc test. P<0.05 was considered to indicate a statistically significant difference.

Results

NGF inhibits MSC apoptosis induced by HG. Flow cytometry showed that there was no significant difference in the apoptosis rate between the LG and LG+NGF groups. Compared with that in the LG group, the apoptosis rate of MSCs in the HG group was significantly increased. NGF was able to significantly inhibit the apoptosis of MSC caused by HG; compared with that in the HG group, the apoptosis rate of MSCs in the HG+NGF group was decreased by 60.98%. TrkA inhibition was able to completely block this effect of NGF (Fig. 1A and B).

The MTT assay indicated that there was no significant difference in the viability rate between the LG and LG+NGF groups. Compared with that in the LG group, the proliferation rate of MSCs in the HG group was significantly decreased. Compared with that in the HG group, the viability rate in the HG+NGF group was increased to 24.5% and the difference was significant. TrkA inhibition was able to completely block this effect of NGF (Fig. 1C).

NGF inhibits the transformation of MSCs into proinflammatory type under HG conditions. As established by numerous studies, MSCs may be divided into a proinflammatory type

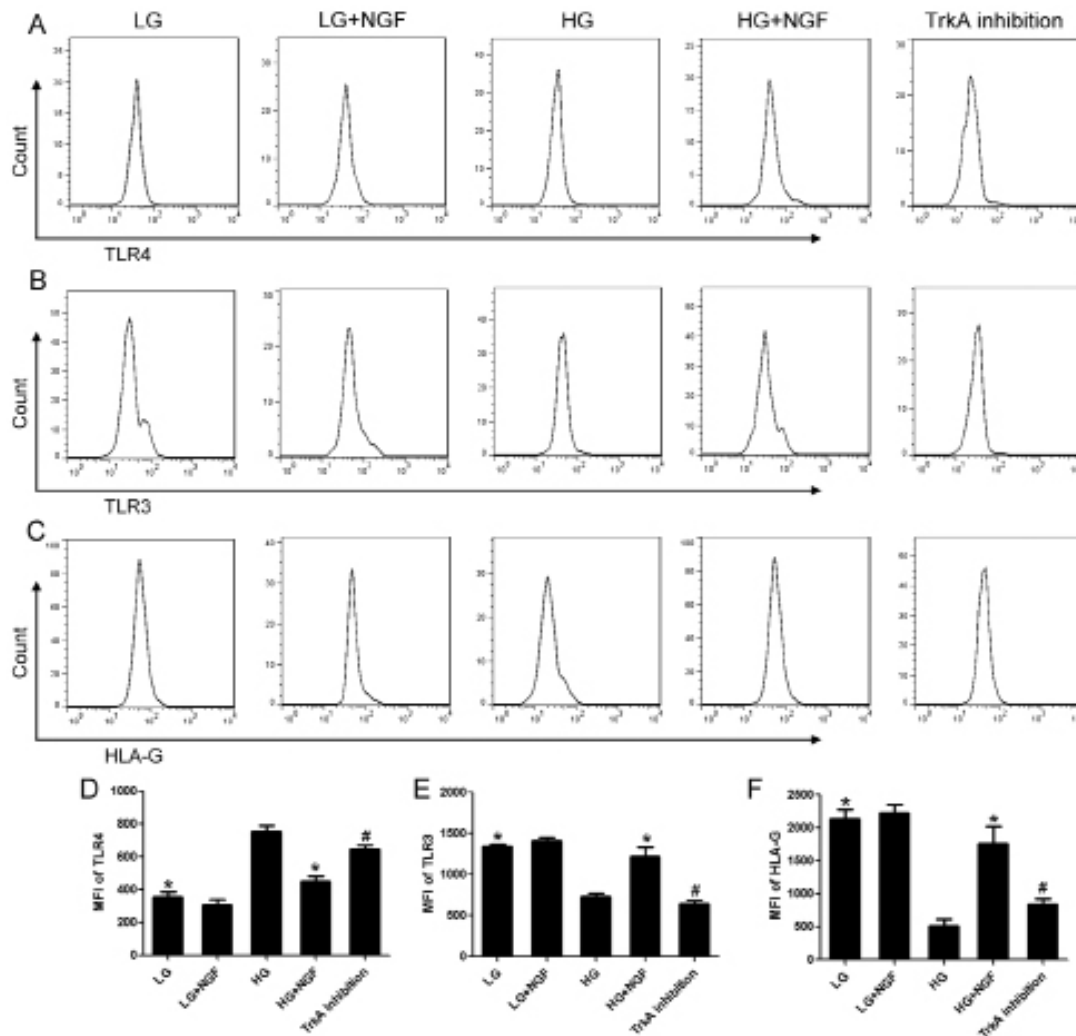


Figure 2. NGF inhibits the transformation of MSCs into the proinflammatory type under HG conditions. (A) TLR4, (B) TLR3 and (C) HLA-G expression was detected by flow cytometry. Quantified levels of (D) TLR4, (E) TLR3 and (F) HLA-G expression. Compared with that in the HG group, the expression of TLR3 and HLA-G in the HG+NGF group increased by 68.27% and 1.41-fold, and the expression of TLR4 decreased by 40.39%. TrkA inhibition completely blocked these effects of NGF. Values are expressed as the mean \pm standard deviation ($n=6$). * $P<0.05$ vs. HG; # $P<0.05$ vs. HG+NGF. TrkA, neurotrophic receptor tyrosine kinase 1; MSC, mesenchymal stem cell; NGF, nerve growth factor; HG, high glucose; LG, low glucose; TLR, Toll-like receptor; HLA-G, human leukocyte antigen G; MFI, mean fluorescence intensity.

and an immunosuppressive type (19,20). Proinflammatory MSCs have high expression of TLR4 and low expression of TLR3 and HLA-G. Immunosuppressive MSCs have high expression of TLR3 and HLA-G and low expression of TLR4. The present results indicated no significant difference in TLR4, TLR3 and HLA-G expression between the LG and LG+NGF groups. HG enhanced TLR4 expression and decreased TLR3 and HLA-G expression. NGF inhibited the transformation of MSCs into the proinflammatory type under HG conditions; compared with that in the HG group, the expression of TLR3 and HLA-G in the HG+NGF group increased by 68.27% and 1.41-fold, respectively, and the expression of TLR4 decreased by 40.39%. TrkA inhibition was able to completely block these effects of NGF (Fig. 2).

NGF promotes the release of paracrine immunosuppressive molecules from MSCs under HG conditions. Compared with that in the LG group, the concentration of TGF- β and IL-10 in the MSC supernatant was significantly decreased in the HG group, while the concentration of TNF- α and IL-6

was significantly increased. NGF promoted the secretion of immunosuppressive molecules from MSCs under HG conditions; compared with that in the HG group, the concentration of TGF- β and IL-10 in the HG+NGF group was significantly increased, while the concentration of TNF- α and IL-6 was significantly decreased. TrkA inhibition was able to completely block these effects of NGF (Fig. 3).

NGF enhances the function of MSCs in inhibiting T cells under HG conditions. The proportion of CD45⁺CD3⁺T cells among lymphocytes of the HG-MSC group was significantly increased compared with that in the LG-MSC group. The proportion of CD45⁺CD3⁺T cells in the HG+NGF-MSC group was 56.42%, with a statistically significant difference compared with that in the HG-MSC group. TrkA inhibition was able to completely block these effects of NGF (Fig. 4).

NGF enhances the function of MSCs to inhibit monocytes/macrophages under HG conditions. In the co-culture experiment, the proportion of CD14⁺ monocytes/macrophages in the

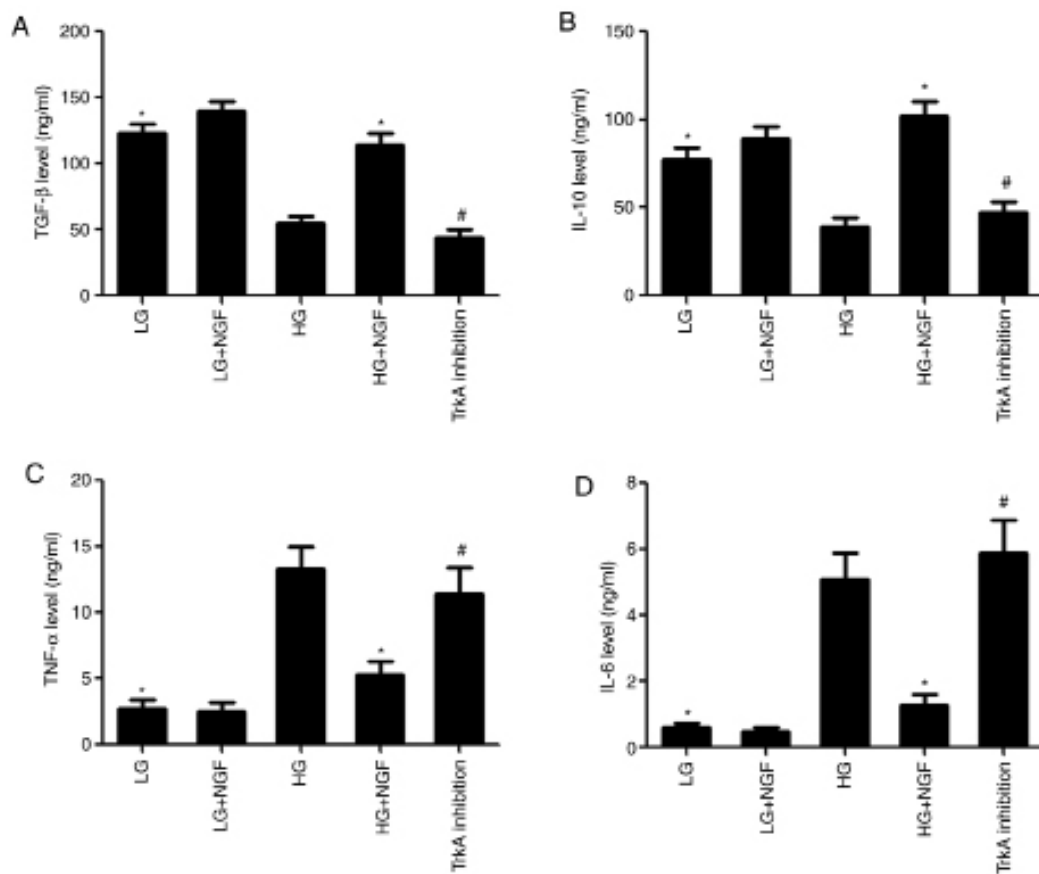


Figure 3. NGF promotes the release of paracrine immunosuppressive molecules from MSCs under HG conditions. The concentrations of (A) TGF- β , (B) IL-10, (C) TNF- α and (D) IL-6 were detected by ELISA. Values are expressed as the mean \pm standard deviation (n=6). *P<0.05 vs. HG; #P<0.05 vs. HG+NGF. TrkA, neurotrophic receptor tyrosine kinase 1; MSC, mesenchymal stem cell; NGF, nerve growth factor; HG, high glucose; LG, low glucose.

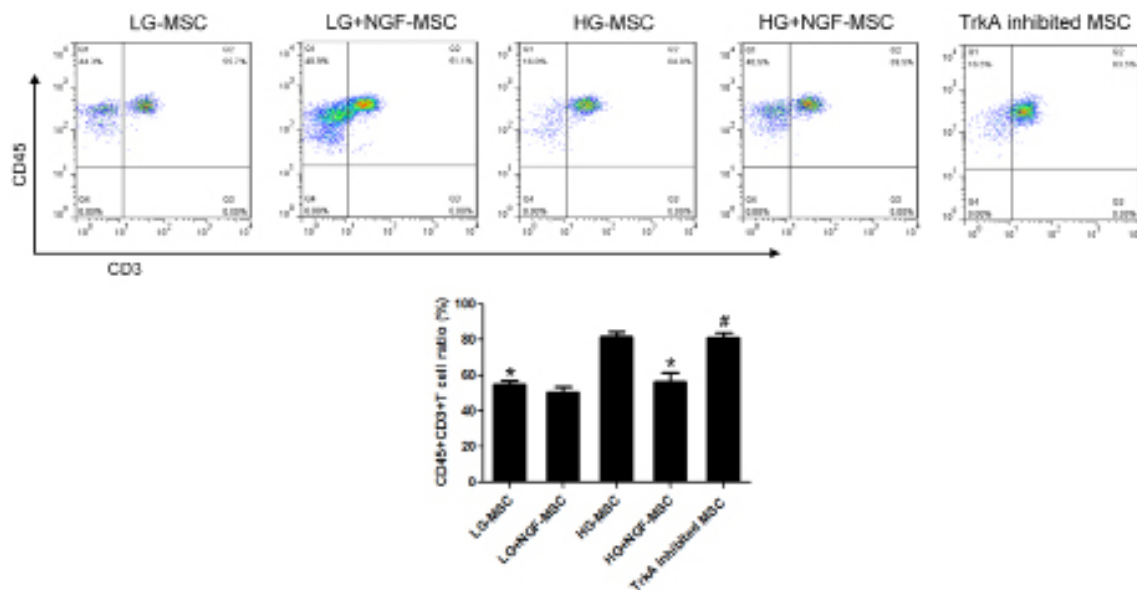


Figure 4. NGF enhances the function of MSCs in inhibiting T cells under HG conditions. The proportion of CD45⁺CD3⁺ T cells in lymphocytes of the HG+NGF-MSC group was significantly decreased compared with that of the HG-MSC group. TrkA inhibition completely blocked these effects of NGF. Values are expressed as the mean \pm standard deviation (n=6). *P<0.05 vs. HG-MSC; #P<0.05 vs. HG+NGF-MSC. TrkA, neurotrophic receptor tyrosine kinase 1; Q, quadrant; MSC, mesenchymal stem cell; NGF, nerve growth factor; HG, high glucose; LG, low glucose.

HG-MSC group was significantly increased compared with that in the LG-MSC group. The proportion of CD14⁺ monocytes/macrophages in the HG+NGF-MSC group was 9.53%,

which was significantly lower than that in the HG-MSC group. TrkA inhibition was able to completely block these effects of NGF (Fig. 5).

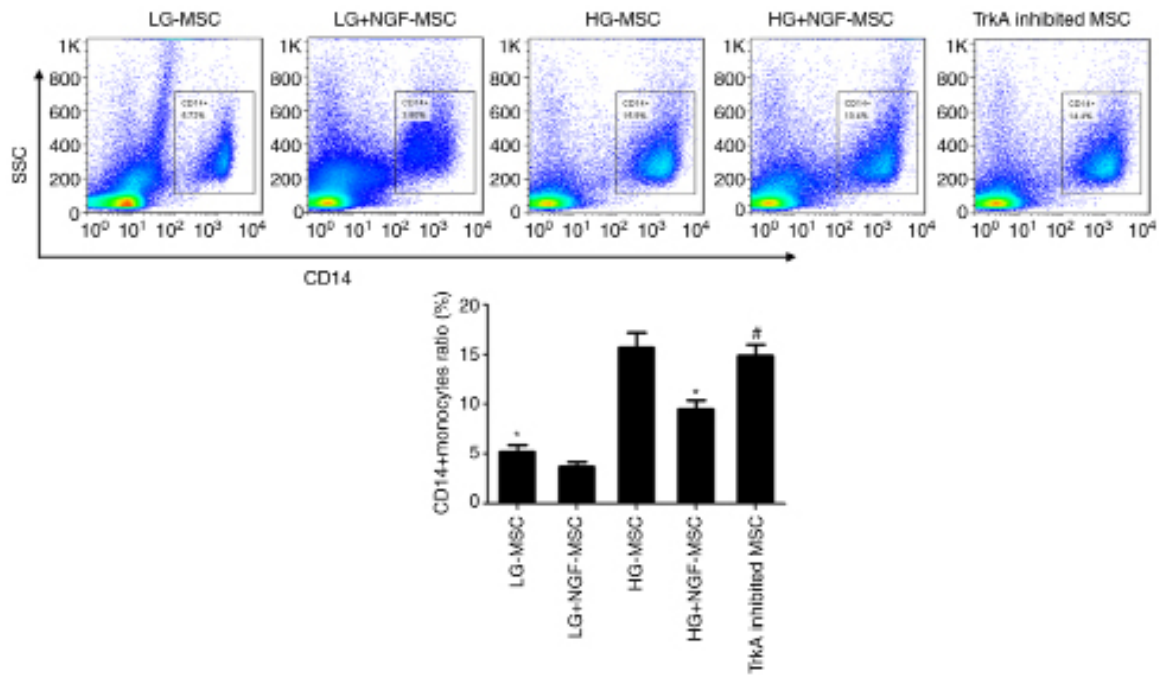


Figure 5. NGF enhances the function of MSCs in inhibiting monocytes/macrophages under HG conditions. The proportion of CD14⁺ monocytes/macrophages in the HG+NGF-MSC group was significantly decreased as compared with that in the HG-MSC group. TrkA inhibition completely blocked these effects of NGF. Values are expressed as the mean \pm standard deviation (n=6). *P<0.05 vs. HG-MSC; #P<0.05 vs. HG+NGF-MSC. TrkA, neurotrophic receptor tyrosine kinase 1; SSC, side scatter; MSC, mesenchymal stem cell; NGF, nerve growth factor; HG, high glucose; LG, low glucose.

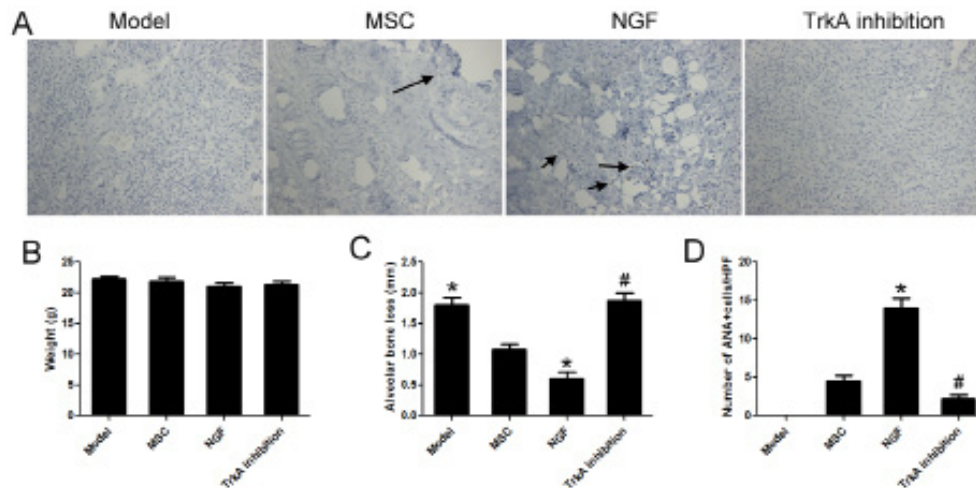


Figure 6. NGF maintains MSC survival in periodontal tissue of diabetic mice. (A) ANA was used to detect transplanted human bone marrow MSCs in periodontal tissue. Arrows indicate ANA⁺ cells (magnification, $\times 100$). (B) There was no significant difference in body weight between the groups. (C) Compared with the MSC group, alveolar bone loss in the NGF group decreased by 43.62%. (D) Compared with the MSC group, the number of transplanted MSCs in the NGF group increased by 2.11-fold. TrkA inhibition completely blocked these effects of NGF. Values are expressed as the mean \pm standard deviation (n=6). *P<0.05 vs. MSC; #P<0.05 vs. NGF. TrkA, neurotrophic receptor tyrosine kinase 1; Q, quadrant; MSC, mesenchymal stem cell; NGF, nerve growth factor; HG, high glucose; ANA, anti-nuclear antibody; HPF, high-power field.

NGF maintains MSC survival in periodontal tissue of diabetic mice. The results of the animal experiment are provided in Fig. 6. There was no significant difference in body weight between the groups (Fig. 6B). Compared with that in the model group, alveolar bone loss in the MSC group was significantly decreased. NGF significantly enhanced the repairing effect of MSCs and alveolar bone loss in the NGF group was decreased by 43.62% compared with that in the MSC group (Fig. 6C). Furthermore, immunohistochemistry with ANA was used to label hMSCs in periodontal tissues. The number of transplanted MSC cells in the NGF group

was 3.11 times that of the MSC group. TrkA inhibition was able to completely block these effects of NGF (Fig. 6A and D). These results indicated that high glucose could damage MSCs, and NGF had a protective effect and reduced MSC death *in vivo*.

NGF enhances the anti-inflammatory function of MSCs in diabetic mice. Immunohistochemical analysis of periodontal tissue indicated that compared with that in the model group, the number of T cells and macrophages was markedly decreased in the MSC group. NGF further inhibited the infiltration of

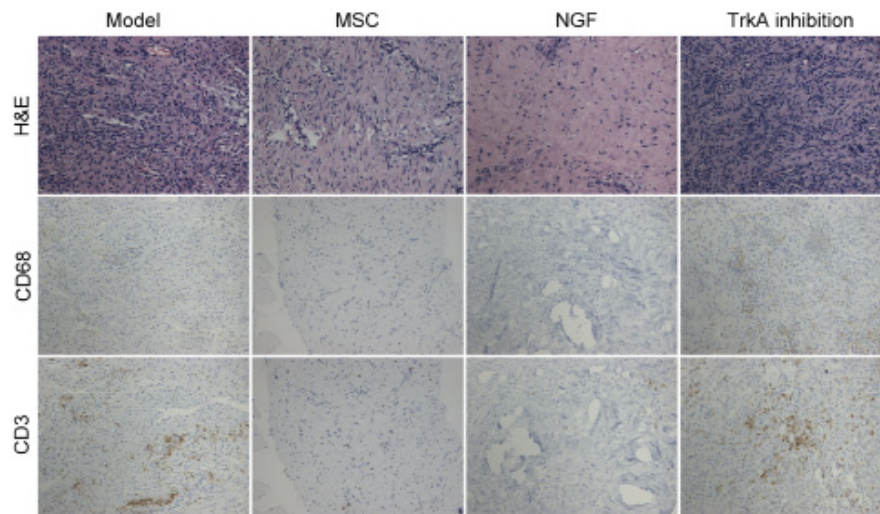


Figure 7. NGF enhances the anti-inflammatory function of MSCs in periodontal tissue of diabetic mice. Compared with that in the diabetes group, the number of CD3⁺ T cells and CD68⁺ macrophages was significantly decreased in the MSC group. NGF further inhibited the infiltration of CD3⁺ T cells and CD68⁺ macrophages. TrkA inhibition completely blocked these effects of NGF (magnification, x100). TrkA, neurotrophic receptor tyrosine kinase 1; MSC, mesenchymal stem cell; NGF, nerve growth factor.

T cells and macrophages. TrkA inhibition blocked these effects of NGF (Fig. 7).

Discussion

Periodontitis is a chronic inflammatory disease of periodontal tissue, with high incidence and recurrent episodes, bringing great pain to patients (21). HG and low immunity make diabetes patients more likely to develop periodontitis. This periodontitis progresses rapidly and is frequently difficult to cure (22). In severe cases, it is even secondary to systemic inflammation, aggravating the condition of diabetic patients (23). In order to study the effect of diabetes on periodontal tissue, a diabetic mouse model was constructed in the present study. The fasting blood glucose in the animals was >16.7 mmol/l for three consecutive times, suggesting that the diabetic mouse model was successfully constructed. In these diabetic mice, the periodontitis model was then constructed. After two weeks, the alveolar bones and periodontal tissues were collected. H&E and immunohistochemical results indicated that a large number of T cells and macrophages were present in periodontal tissues. MSCs have important functions, such as tissue repair and anti-inflammatory effects. The present results indicated that MSC transplantation reduced the infiltration of inflammatory cells and the expression of inflammatory molecules in periodontal tissues. There are two main mechanisms by which MSCs exert their anti-inflammatory role. Immunosuppressive molecules that are highly expressed on the MSCs are able to inhibit T cells and macrophage-mediated immune responses through cellular contact. In addition, MSCs paracrine numerous anti-inflammatory molecules to inhibit local inflammatory responses (24).

HG and high oxidative stress may damage the function of transplanted stem cells in patients with diabetes, which makes the effect of MSC therapy unsatisfactory (25). The present results indicated that HG increased MSC apoptosis and inhibited MSC paracrine function. Therefore, it is of great significance to identify novel targets to protect MSCs in

diabetes. Peripheral neuropathy is an important complication of diabetes, which may cause itching, abnormal sensation and diabetic ulcers. Promoting nerve injury repair may effectively treat diabetic peripheral neuropathy (26). NGF has the function of promoting nerve differentiation and axon growth and has been used in the clinic. In recent years, numerous studies have confirmed that NGF is able to protect cells and repair tissues (27,28). The present study indicated that NGF inhibited apoptosis of MSCs caused by HG and restored their damaged paracrine function. The animal experiment indicated that pretreatment with NGF significantly improved the survival rate of MSCs in diabetic periodontal tissues and enhanced their therapeutic effect on periodontitis. HG-induced damage to MSCs may also be mediated by other mechanisms, such as hyperosmosis, high oxidative stress and DNA damage. In the present study, these aspects were not explored, which is a limitation of the present study.

Numerous studies have indicated that MSCs may have two different phenotypes. Waterman *et al* (19) divided MSCs into proinflammatory MSCs and immunosuppressive MSCs based on their expression of TLR3 and TLR4. Svobodova *et al* (20) also indicated that MSCs with different phenotypes affect the differentiation of naive T cells into regulatory or proinflammatory T cells. To better simulate the transplantation of hMSCs into mice in animal experiments, mouse lymphocytes and hMSCs were co-cultured *in vitro* in the present study. Under physiological conditions, MSCs have a strong anti-inflammatory effect (immunosuppressive MSCs). However, in the present study, when MSCs were in an HG environment, immunosuppressive MSCs were converted to proinflammatory MSCs and the expression of immunosuppressive protein and secretion of anti-inflammatory molecules were significantly decreased, while the secretion of pro-inflammatory molecules was significantly increased. The *in vivo* experiments of the present study demonstrated that NGF was able to inhibit the conversion of MSCs to the proinflammatory type in diabetes and further decreased the infiltration of T cells and macrophages in periodontal tissues.

TrkA is the major high-affinity receptor of NGF and has an irreplaceable role in the growth and development of the nervous system and the maintenance of neuronal characteristics (29). The combination of NGF and TrkA may further activate the MAPK signaling pathway and promote stem cell proliferation and differentiation (30,31). Numerous studies have indicated that the TrkA receptor may be detected on MSCs, and TrkA receptor expression is significantly increased under HG and ischemic conditions (32-34). The present study indicated that mainly through the TrkA receptor, NGF protected MSCs against HG damage and enhanced their anti-inflammatory effect in diabetes. GW441756 is a highly specific inhibitor of TrkA. Numerous studies have proved that GW441756 has a good effect on TrkA inhibition (35,36), so it was applied in the present study. The present results demonstrated that TrkA receptor inhibition completely blocked these functions of NGF.

In conclusion, the present study indicated that NGF is able to enhance the therapeutic effect of MSCs in diabetic periodontitis by protecting the cells and promoting the transformation of MSCs into the immunosuppressive type. The present study provided a novel agent for stem cell therapy for diabetic periodontitis.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

JP, YL and SW drafted and revised the manuscript. JP and YL designed and performed most of the experiments. SW performed the cell experiments. YX and BL performed the animal experiments. JP and YL confirm the authenticity of all the raw data. All authors discussed the results and approved the final submitted version. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The animal protocol was approved by the Ethics Committee of The Air Force Characteristic Medical Center (Beijing, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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