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Original Article

Effect of autologous bone marrow transplantation combined with SDF-1 alpha on diabetic peripheral neuropathy

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Abstract: Aim: The primary purpose of this study is to investigate the therapeutic effect of autologous bone marrow transplantation combined with stromal cell-derived factor-1α (SDF-1α) injected locally in a rat model of diabetic peripheral neuropathy. Methods: 48 male SPF SD rats (6 weeks old, average body weight 180-200 g) were randomly allocated to normal group (NC, n=8) and type 1 diabetes model group (n=38). The rat model of type 1 diabetes was built by administering a single intraperitoneal injection of streptozotocin (STZ, 60 mg/kg), and the established model (n=27) of rats was casually assigned to three groups: diabetes group (MD, n=10), diabetic autologous bone marrow transplantation group (DB, n=10), and diabetic autologous bone marrow transplantation combined with SDF-1α treatment group (DBS, n=7). After 4 weeks of bone marrow transplantation, we determined the behavioral index of rats (algesia and thermesthesia), sensory nerve conduction velocity and motor nerve conduction velocity. Sciatic nerve morphology was observed by light and electron microscopy, and the mRNA level of bFGF and Gli was assayed by real-time PCR. Results: Serious peripheral neuropathy occurred in type 1 diabetic rats, whose nerve conduction velocities were significantly lower than normal ones. Moreover, we observed reduced pain threshold, serious damaged sciatic nerve and swelling and apomorphosis of Schwann cells. In addition, the level of neurotrophic factor secreted by the sciatic nerve was relatively insufficient. After receiving autologous bone marrow transplantation, neuropathy in DB and DBS showed significant improvement. Conclusion: Autologous bone marrow transplantation can improve diabetic peripheral neuropathy, the effect of which can be increased by local administration of SDF-1α from this study.

Keywords: Autologous bone marrow transplantation, diabetic peripheral neuropathy, neurotrophic factor, SDF-1α

Introduction

Diabetic neuropathy (DN) is a heterogeneous spectrum of disorders, which results in impairment of somatic and autonomic nerves. Chronic hyperglycemia has been proved to be the major culprit in the initiation and development of extremely complex pathophysiology underlying DN, associated with high disability and mortality. Current studies addressed the pathogenic mechanisms underlying diabetic neuropathy from three main aspects: abnormal neurocyte metabolism, vasa nervorum disease and reduction of neurotrophic factors. Among these respects, microvascular disease of vasa nervorum was considered as the basis of pathological development that links DN [1]. Kusano KF et al. found that the lack of growth factors locally, such as vascular endothelial growth factors (VEGFs), contributes to the reduction of capillaries [2, 3], which is also involved in the pathophysiology of DN. Unfortunately, the effective and specific therapy is still not available especially in the presence of great risk of DPN.

The only proven strategy to treat DN from mechanic aspect is strict glycemic control, although symptomatic relief are commonly used to improve blood circulation. Leinninger GM et al. demonstrated that lack of neurotroph-

Table 1. Comparison of metabolic profiles between the four experimental groups

	NC	MD	MB	MBS
BW (g)	574.2±19.91	247.6±14.12**	263±14.68**	284.2±8.27**
FBG (mmol/L)	6.22±0.38	29.60±1.21**	30.57±1.00**	30.04±1.26**
GSP (mmol/L)	0.862±0.028	4.507±0.178**	4.495±0.221**	4.460±0.202**

Notes: Data are expressed as mean ± S.E.M. NC, normal control; MD, model of diabetes; MB, autologous BM transplantation; MBS, autologous bone marrow transplantation combined with local injection of SDF-1α. **P<0.01 vs. NC.

ic factors was another important mechanism in aggravation of DN [4], which recently has been found to exert protective and regenerative effects on peripheral neurons. Hence, a possibly new approach to get around DPN treatment is to combine the improvement of angiogenesis with the increase of local neurotrophic factors.

As we know, myelopoietic cells, erythropoietic cells, megakaryocytes and lymphocytes arise in the bone marrow (BM), which also contains poorly differentiated hematopoietic stem cells with the ability to differentiate into a variety of cell types [5-11]. The effect of BM stem cells on the revascularization of diabetic animals was significantly weaker than that of healthy rats. Therefore, improving the survival, proliferation and function of BM stem cells in target organs is critical for assuring the therapeutic efficacy of autologous BM stem cell transplantation in diabetic rats.

The stromal-cell-derived factor (SDF)-1α, also known as CXCL12, that is the strongest chemotactic factor for hematopoietic stem/progenitor cells (HSPCs), plays a critical role in mobilization and migration of CD34+ HSPCs and endothelial progenitor cells (EPCs) via activating unique receptor CXCR4, which benefits the activation of Akt and eNOS [12]. Besides, it also works as a fundamental part in development and repair of nervous system, where SDF-1α reduces apoptosis and promotes the secretion of growth factors by positive feedback in BM stem cells, and thus encourages ischemia-induced angiogenesis and peripheral nerve repair.

This study explored the effect of autologous bone marrow transplantation (ABMT) on DPN with combination of local injection of SDF-1α, and investigated the potential function on generation of local neurotrophic factors in STZ-induced DPN rats to lay foundation for further clinical application.

Materials and methods

Specimen preparation

Animals

Forty-eight 6-week-old male SD rats (weighing 180-200 g) were purchased from Hunan SJA Laboratory Animal Co. (HNASLKJ 20120139).

Modeling

The experimental rats were anesthetized and then injected with a single dose of 60 mg/kg STZ in 100 mM sodium citrate buffer at pH 4.2. After the 3 days and 7 days STZ, fasting blood glucose (9 am to 3 pm) was measured from the tail vein using a glucometer. Mice with plasma glucose concentrations of 16.7 mmol/L were selected as the STZ-induced diabetic rats. Following 8 weeks, the modeled rats exhibited a classic phenotype of diabetic peripheral neuropathy [13].

Grouping

All SD rats had free access to water and diet with a laboratory standard for 1 week, and the mice were randomly allocated to normal control group (n=8) and experimental group (n=40). With 12 h of fasting (8 pm to 8 am), the experimental rats were anesthetized and then injected with a single dose of 60 mg/kg STZ in 100 mM sodium citrate buffer, pH 4.2. The control mice were administrated with an injection of citrate buffer. The STZ-injected rats after 3 and 7 days, fasting blood glucose (9 am to 3 pm) was measured from the tail vein using a glucometer. Mice with plasma glucose concentrations of 16.7 mmol/L were selected as the STZ-induced diabetic group (n=27). Eight weeks later, diabetic rats were randomly divided into three groups: model of diabetic group (MD, n=10), the diabetic autologous bone marrow transplantation group (DB, n=10), and the diabetic autologous bone marrow transplantation combined with SDF-1α local injection group (DBS, n=7).

Autologous BM collection and transplantation: After intraperitoneal anesthesia with 10% chloral hydrate, the left knee was disclosed without

Table 2. PWT and PWL response to mechanical and thermal stimuli at bilateral central plantar surface of T1DM rats

	NC-L	NC-R	MD-L	MD-R	MB-L	MB-R	MBS-L	MBS-R
PWT (g)	39.54±1.83	39.24±1.80	30.30±1.58**	30.10±1.61**	30.50±2.05**	37.00±1.57*,##	29.80±1.70**	38.12±2.82##
PWL (s)	22.13±2.35	21.48±1.81	11.26±2.77**	11.93±2.83**	12.28±0.94**	18.18±1.52**,##	12.33±1.042**	20.53±2.47##

Notes: Data are expressed as mean ± S.E.M. L, left; R, right. PWT, paw withdrawal threshold; PWL, paw withdrawal latency; *P<0.05 vs. the homolateral plantar surface of NC; **P<0.01 vs. the homolateral plantar surface of NC. ##P<0.01 vs. the left plantar surface of the same rat.

Table 3. Comparison of PWTD and PWLD between the right hind paw and left hind paw in a single rat.

	NC	MD	MB	MS
PWTD	-0.080±0.996	0.200±2.060	6.556±0.800##	8.308±1.293##,&&
PWLD	0.643±1.144	0.671±1.394	5.910±1.922##	8.206±2.620##

Notes: PWTD, paw withdrawal threshold difference between the left and right hind paws; PWLD, paw withdrawal latency difference between the left and right hind paws; ##P<0.01 vs. MD; &&P<0.01 vs. MB.

severance of the muscles and ligaments, and then a sterile 7-gauge lumbar puncture needle was used to screw into the marrow cavity of tibial shaft. Approximately 0.2 mL of BM was aspirated from each rat, mixed with 50 μl heparin, and then injected into six selected points of right brachial quadriceps, biceps and soleus using a microinjector (40 μl per point).

SDF-1α injection: Murine SDF-1α was dissolved with 0.4% BSA solution and then injected into the six above-mentioned points after autologous BM transplantation (10 μl per point).

Intervention: Autologous BM collection was conducted in the unilateral tibial shaft of all rats, and then injected into another tibial shaft of the rats of MB and MBS groups according to section 1.2. The rats of NC and MD groups were treated with 0.2 ml saline to replace autologous BM, while the rats of MBS group were injected with SDF-1α from section 1.3.

Determination of blood glucose and glycated serum albumin

On the experimental day, food was removed at 8 am and blood was sampled from the tail vein after 6 hours. Blood glucose was checked using a glucometer (Bayer, Leverkusen, Germany) with STZ injected rats after 3 and 7 days.

Glycosylated serum protein (GSP), a parameter reflecting average blood glucose in the past 2-4 weeks, was detected after 4 weeks of autologous bone marrow transplantation using a commercial kit.

Ethological methods

Mechanical hyperalgesia

Mechanical hyperalgesia was determined by the electronic von Frey filament test, while the rats were placed for 15 mins on the wire mesh floor for adaptation before measurement. A standardized probe was then applied vertically to the central plantar surface of the hind paw from bottom to upward until a clear withdrawal was observed. The response magnitude expressed in "g" was evaluated as paw withdrawal threshold (PWT) registered automatically by the electronic device. Besides, the left and right paws were measured at least three times for each paw to find the paw withdrawal threshold from mechanical stimulation.

Thermal hyperalgesia

Thermal hyperalgesia was measured by a plantar test initiated after 15 mins, while a three-chamber container was used to separate rats placed on the glass base. In the condition of randomized-blind treatment, the paw withdrawal latency (PWL) was recorded that responds to a thermal stimulus at central plantar surface (a heat source that radiated a light beam, 4 mm × 6 mm). PWL was defined as the time from the start of the beam light to the paw withdrawal reflex (the measured reaction time 0.1 s). The tests were repeated at least three times with 1 min interval between two tests, and performed for both paws.

Electroneurophysiological detection

Measurement of nerve conduction velocity was used to evaluate the function of myelinated nerve fibers. Rats were anesthetized with chloral hydrate (100 g/L) and placed on a warming pad to maintain body temperature at 37°C. A surgery was then conducted on the right hind limb of the anesthetized rats to expose the sci-

Table 4. Bilateral sciatic nerve MNCV and SNCV in all groups

	NC-L	NC-R	MD-L	MD-R	MB-L	MB-R	MBS-L	MBS-R
SNCV (m/s)	49.21±2.87	49.29±3.90	37.63±2.37**	36.43±4.19**	36.63±3.35**	42.31±4.99*,#	36.80±3.80**	44.02±5.54#
MNCV (m/s)	58.37±2.54	59.88±3.18	45.97±3.27**	46.27±3.72**	43.68±4.30**	50.55±4.16**,#	45.88±3.01**	53.61±3.47*,##

Notes: Data are expressed as mean ± S.E.M. L, left; R, right. SNCV, sensory nerve conduction velocity; MNCV, motor nerve conduction velocity; *P<0.05 vs. the homolateral sciactic nerve of NC; **P<0.01 vs. the homolateral sciatic nerve of NC. #P<0.05 vs. the left sciatic nerve of the same rat; ##P<0.01 vs. the left sciatic nerve of the same rat.

Table 5. Comparison of MNCVD and SNCVD between the right sciatic nerve and left sciatic nerve in a single rat

	NC	MD	MB	MS
SNCVD	0.084±1.864	-1.209±2.215	5.685±3.673##	7.224±6.555#
MNCVD	0.600±2.930	0.303±4.280	6.872±0.972#	7.730±4.430#

Notes: Data are expressed as mean ± S.E.M. SNCVD, sensory nerve conduction velocity difference between the left and right sciatic nerves; MNCVD, motor nerve conduction velocity difference between the left and right sciatic nerves; #P<0.05 vs. MD; ##P<0.01 vs. MD.

atic nerve. The sciatic nerve was stimulated at the ischial fossa and ankle, where two fine needle electrodes were inserted 2 mm apart to detect the conduction. Throughout the experiment, the surface of the hind limb was kept at 30°C.

Motor nerve conduction velocity (MNCV)

The sciatic nerve was stimulated via electrodes previously inserted with constant-current (10-20 mA) square-wave pulses (40 μs) to generate compound muscle action potentials. Three pairs of latency-of-M waves were recorded and averaged. The average latency difference (ALD) between the initial onset and maximum negative peaks was defined as conduction time (CT) between the two sites. MNCV was computed by dividing the distance between the stimulating electrodes by ALD.

Sensor nerve conduction velocity (SNCV)

Again, the sciatic nerve was stimulated via the electrodes previously inserted with constant-current (2 mA) square-wave pulses (40 μs) to evoke an H-reflex. Meanwhile, 6 pairs of latency were recorded and the minimal latency difference (MLD) between the two sites was calculated. SNCV was computed with dividing the distance of the stimulating electrodes by MLD. The stimulus was digitized and captured with RM6240 multi-channel signal collection system.

Histology detection

Fragments of the sciatic nerves (1-2 cm) were prepared and fixed in 25 g/L glutaraldehyde solution overnight at +4°C. The samples were post-fixed with 10 g/L osmium tetroxides at 4°C for 1 hour, and subsequently dehydrated and embedded in resin and then put in araldite mixture. Blocks were placed at 60°C for 48 hours to polymerize. Semi-thin sections were stained with 5% uranyl acetate and lead citrate, and then viewed on the electron microscope.

Real-time PCR

Total RNA of the sciatic nerves was extracted according to the instruction provided by Tarkar Co. and the concentration of RNA was measured using an ultraviolet spectrophotometer. Total RNA was used to synthesize cDNA in a 10 μl reverse transcription system, which was applied to the target gene amplification with real-time PCR in a 20-μl system. The amplification products (4 μl) were used to conduct electrophoresis in 2% agarose gel until separation of the electrophoretic band, subsequently observed under UV light and analyzed using v ILBERLOURMAT imaging system.

Statistical analysis

Data are presented as mean ± S.E.M. Statistical analysis was performed by the SPSS16.0 program. The differences between the two groups were examined forstatistical significance using t-test, while the ANOVA analysis was used to compare the parameters (such as sensory nerve conduction velocity, motor nerve conduction velocity, etc.) and groups. *P*-values <0.05 were considered statistically significant.

Results

Characteristics of STZ-induced T1DM rats

Among all SD rats, body weight at baseline presented no significant difference. However, a single dose of STZ injection destroyed most of

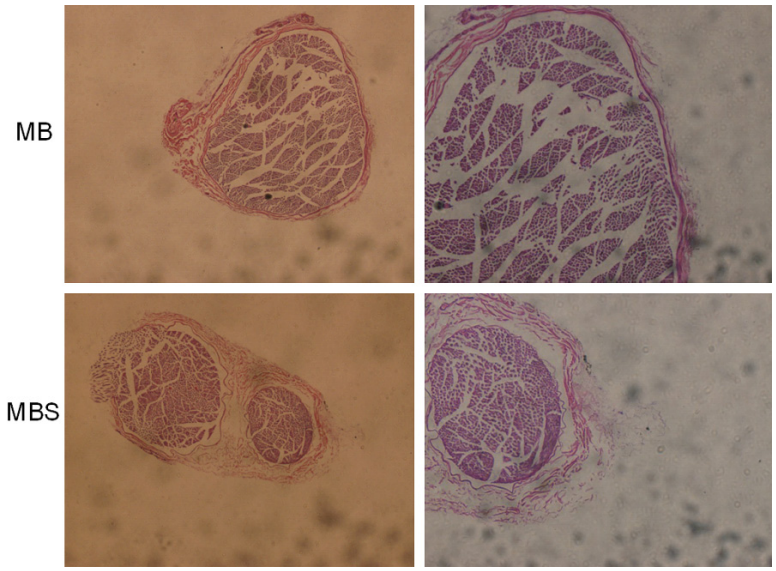

Figure 1. Light micrographs of sciatic nerve in the NC, MD, MB, and MBS groups. NC, normal control group; MD, model of diabetes group; MB, autologous bone marrow transplantation group; MBS, autologous bone marrow transplantation combined with SDF-1α local injection group.

β-cells in the islet, and led to the absolute lack of insulin secretion, resulting in the formation of rat models of type 1 diabetes that characterized hyperglycemia, polydipsia, polyuria, polyphagia, and weight loss.

After intervention with autologous BM or combined with SDF-1α for 4 weeks in T1DM rats, body weight (BW), fasting blood glucose (FBG) and glycosylated serum protein (GSP) were measured, and the results were shown in **Table 1**. Compared with the NC group, T1DM rats exhibited significantly reduced BW, continuously elevated FBG (average of 30 mmol/L) and increased GSP (P<0.01), while the marked difference was not generated between MD, MB and MBS groups (P>0.05), indicating that intervention with autologous bone marrow transplantation or combined with SDF-1α exerts the feebly therapeutic effect on BW, FBG and GSP.

Effect of ABMT combined with SDF-1α on small myelinated and unmyelinated nerve fibers in T1DM rats

Mechanical and thermal hyperalgesia were performed to test the function of small myelinated and unmyelinated nerve fibers. As shown in **Table 2**, 4 weeks after the intervention, rats in the MD group indicated a lower PWT and PWL response to repetitive mechanical stimuli than that of the NC group (P<0.01), while PWT and PWL were similar between the two hind paws and remained stable during repeated testing (P> 0.05). However, ABMT treatment markedly increased PWT and PWL, demonstrated by elevated PWT and PWL in the right hind paw (ABMT administration) versus the left hind paw (no ABMT) (P<0.01), for instance, whose PWT and PWL presented parallel alteration with that of the MD group (P>0.05). While PWT and PWL of the right hind paw in the MBS group were noticeably increased than that of the MB group and close to the level of the NC group (P> 0.05). In addition, as shown in **Table 3**, ABMT combined with SDF-1α was more effective than ABMT alone in restoring PWT and PWL, as suggested by significantly greater paw withdrawal threshold/latency difference (PWTD/PWLD) (8.308 g vs. 6.556 g in PWT; 8.206 vs. 5.910 in PWL; P<0.01).

Effect of ABMT combined with SDF-1α on sciatic nerve conduction velocity

MNCV and SNCV were conducted to test the function of myelinated nerve fibers, as shown in **Table 4**. In the group of T1DM rats, conductance of motor nerve and sensory nerve were significantly reduced by approximately 22.0% and 21.9%, respectively, in the diabetic group versus the NC group. In contrast, MNCV and SNCV were reversed by 15.7% and 10.0% in the right hind paw of ABMT-treated rats (P<0.05) and presented no difference between the other hind paw of ABMT-treated rats and MD. In addition, the right sciatic nerves of MNCV and SNCV in the MBS group were elevated (by 16.8% and 12.2% vs. MD), which was similar to that of the control group (P>0.05) and demonstrated an increase tendency compared with that of MB.

As shown in **Table 5**, motor/sensory nerve conduction velocity differences (MNCVD, SNCVD) between the right sciatic nerve and the left sciatic nerve in MB and MBS groups, were more significant compared with MB (P<0.01-0.05).

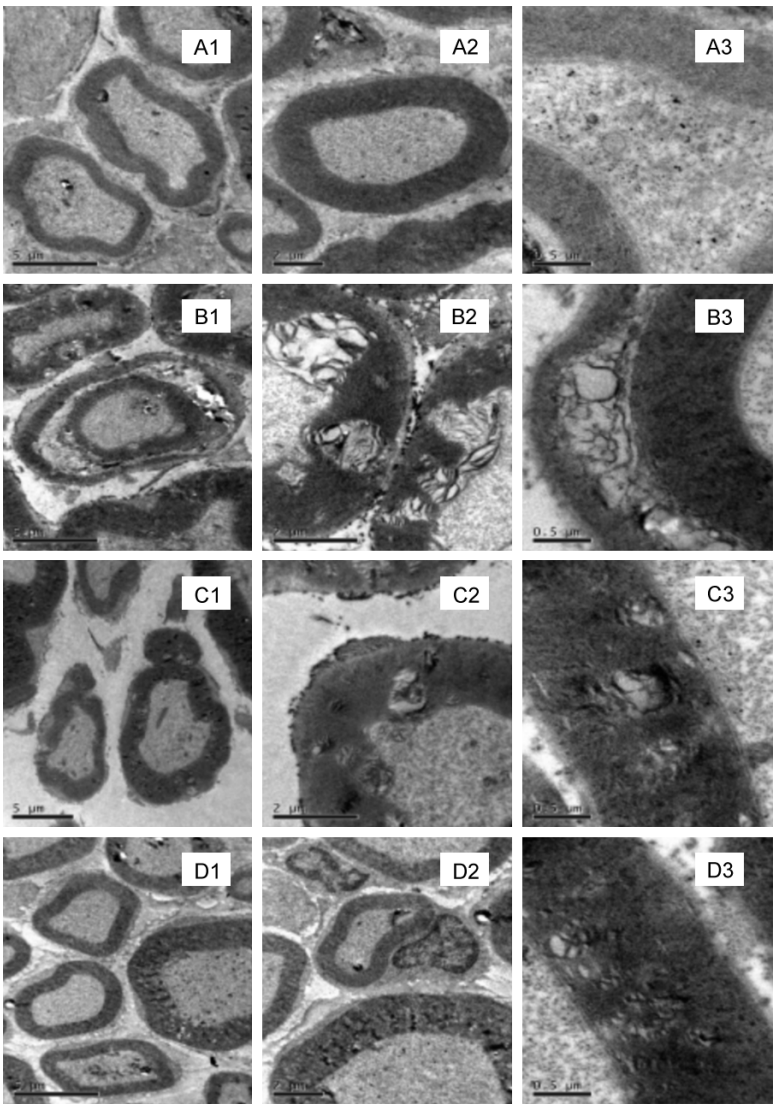

Figure 2. Electron micrographs of sciatic nerve in the NC, MD, MB, and MBS groups. The micrograph of the right sciatic nerve was shown as follows: NC in A1, A2 and A3; MD in B1, B2 and B3; MB in C1, C2 and C3; MBS in D1, D2 and D3. Scale bar: 5 μm, 2 μm, or 0.5 μm.

An increased tendency can be observed in the MBS group than in the MB group.

Effect of ABMT combined with SDF-1α local injection on sciatic nerve morphology in T1DM rats

Light microscope

The morphology of control group revealed insignificant pathology in light microscopy examination, as shown in **Figure 1**, sciatic nerve fibers were closely organized and uniformly distributed. Moreover, the perineurium was also not altered in staining quality and the half-moon Schwann's cells were clearly observed, with ruled capillary loops of morphological and smooth capillary wall.

Severe pathologic scenes were evident in the diabetic samples in light microscope views. Loosely organized nerve fibers, thinner myelin sheath with uneven density, and lower density plaque area were observed. Capillary walls thickened wildly accompanied by occasionally irregular, or even blocked areas, with swelled and deformed capillary endothelial cells. Fiber axons were narrowed and certain parts even blocked completely.

ABMT-treated rats still presented loosely organized sciatic nerve fibers and thinner myelin sheath with uneven density. In addition, capillary walls were thickened and even blocked with swollen and deformed capillary endothelial cells. However, compared with rats in MD group, an increased amount of capillary loops and significantly alleviated pathology canbe observed in ABMT-treated rats.

Treatment with ABMT combined with SDF-1α protected T1DM rats from sciatic nerve pathology. The severe impairment in sciatic nerve fibers and capillary loops was considerably reversed in the part, as demonstrated by more closely organized fibers and elevated amounts of capillary loops than that of MB.

Electron microscope

As shown in **Figure 2**. In control group, the nerve fibers were uniformly and densely packed and regularly ruled, lamellae of which was shaped into concentric circles. Axon presented swelling without shrinkage, where neurofilament and microtubules were aligned, while swelling was not observed in Schwann cells as

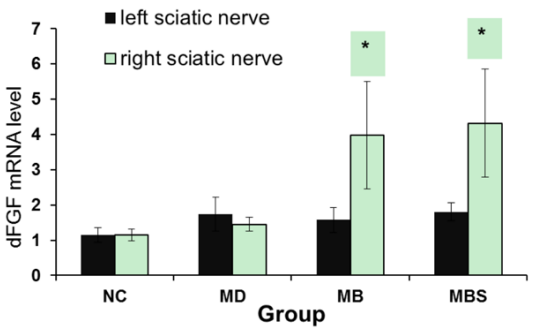

Figure 3. Relative expression of b-FGF mRNA in the sciatic nerve of all groups. Data are expressed as mean ± S.E.M. *P<0.05 vs. the left sciatic nerve of the same rat; ##P<0.01 vs. the left sciatic nerve of the same rat.

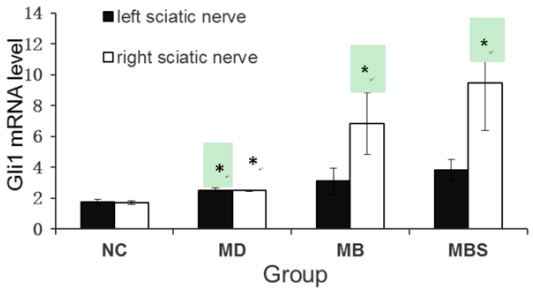

Figure 4. Relative expression of Gli-1 mRNA in the sciatic nerve of all groups. Data are expressed as mean ± S.E.M. *P<0.05 vs. the homolateral sciactic nerve of NC; **P<0.01 vs. the homolateral sciatic nerve of NC. #P<0.05 vs. the left sciatic nerve of the same rat; ##P<0.01 vs. the left sciatic nerve of the same rat.

well as mitochondria within the axon. The ridge of inner membrane of mitochondria was clearly visual. There was rarely any myelin protrusion.

Sciatic nerves of diabetic rats under the electron microscope disclosed damaged myelin configuration, myelin protrusion, lamellous separation, neurofilament, neurotubulous accumulation, derangement and several bubble-shaped defects in myelinated axons. Schwann cells and mitochondria within the axon were widely swollen or broken, and the inner membrane ridge of the mitochondria were disappeared.

ABMT-treated rats also had damaged myelin configuration, myelin protrusion, and lamellous separation, but the degree of pathology was appreciably ameliorated. In addition, the number of bubble-shaped defects and neurofilament and neurotubulous accumulation and derangement was decreased partially. The inner mitochondria membrane ridge was still ruled. Taken together, demyelination and pathology in the axon of the sciatic nerve were relatively lighter than that of the MD group.

In the MBS group, myelinated nerve fibers existed a complete lamellar structure with a smaller density and uniformity. Occasionally bubble-shaped defects, lamellous separation, neurofilament, and neurotubulous derangement were observed, but the inner mitochondria membrane ridge within the axon remained in its original shape. Overall, demyelination and axonal lesions persisted, and the amelioration of sciatic nerve pathology was substantial compared with MD and MB.

Effect of ABMT combined with SDF-1α local injection on neurotrophic factors, b-FGF and Gli-1

b-FGF and Gli-1 are two representative neurotrophic factors with activity in promoting blood vessel growth, referred as vascular nerve factors with other similar growth factors. **Figure 3** implies that even though suffering nerve injury, the transcription of related neurotrophic factors in DPN rats was relatively insufficient without any significant increase compared with NC (P>0.05). In contrast, whether treated with ABMT alone or combined with SDF-1α local injection, the b-FGF mRNA level in the right sciatic nerve of MD, MB and MBS groups was expressively elevated by 27%, 249%, and 278%, respectively. In addition, transcription of b-FGF in the right sciatic nerve of MB and MBS groups was higher than that of the left sciatic nerve (by 139% and 153%). There did not exist the significant difference of b-FGF mRNA level, although there was an increasing tendency in the therapeutic efficacy of DPN between MB and MBS groups. **Figure 4** suggests that even in the presence of nerve injury, the transcription of Gli-1 in DPN rats was relatively insufficient compared with NC (P>0.05). However, the Gli-1 mRNA level in the right sciatic nerve of MD, MB and MBS groups was significantly elevated by 47%, 305%, and 462%, respectively, and it was not dependent on treatment with ABMT alone or combined with SDF-1α local injection. In addition, transcription of Gli-1 in the right sciatic nerve of both MB and MBS groups was higher than that of the left sciatic nerve (by

136% and 157%). The significant difference of Gli-1 mRNA level was not observed, although there was an increase in the therapeutic efficacy of DPN between MB and MBS groups.

Discussion

In this study, after stable establishment of diabetic peripheral neuropathy in rats, the left leg tibia BM was transplanted into the right brachial biceps brachii muscle, quadriceps, soleus muscle and combined with SDF-1α multi-pointed injection. Through the animal behavior experiment, we found that the mechanical hyperalgesia in the rats was improved after BM transplantation, with significantly improved temperature sensation and the enhanced effects with SDF-1 combined. This clearly demonstrated that the autologous bone marrow transplantation combined with SDF-1 could improve the function of peripheral unmyelinated and small myelinated nerve fiber in rats. We can draw a conclusion from the measurement results of the nerve conduction velocity of the sciatic nerve in the rats that autologous bone marrow transplantation and locally administered multi-pointed SDF-1α injection can significantly progress the sciatic nerve motor nerve and sensory nerve conduction velocity, suggesting that this strategy can enhance the rat peripheral large myelinated nerve fiber function. Using a transmission electron microscope to observe the ultrastructure of sciatic nerve, we confirmed that autologous bone marrow transplantation combined with SDF-1α can effectively recover the sciatic nerve in rats: the fiber structure of lamellar structure returned to concentric, dense, reduced cavitation, rare phenomenon of detachment, disappearance of Schwann cell edema and restoration of mitochondrial structure. The recovery of the structure is the basis of improved function.

The above results indicated that we obtained inspiring therapeutic effects by the BM transplantation combined with SDF-1α strategy, to a certain extent, of improving lower limb blood supply and sciatic nerve structure and function in diabetic rats. As widely known, many kinds of BM cells exist, including erythroid, myeloid, lymphoid and mononuclear cell system. A review presented that a variety of BM cells can improve DNP [14], in which the BM mononuclear cells are the main functional cells, including lymphocytes, hematopoietic stem/progenitor cells, endothelial progenitor cells and mesenchymal stem cells.

First, the BM-derived endothelial progenitor cell is one of the earliest and most studied (endothelial progenitor cell, EPC). In 1997, Asahara et al. [15] first found the existence of EPC in adult peripheral blood, which has opened the vascular regenerative cell therapy time. EPC mainly exists in the BM, umbilical vein blood and peripheral blood in small amounts. Since EPC has the ability of proliferation, directed migration, settlement to the ischemic region and involved angiogenesis, all these characteristics make it become the preferred target cells for gene therapy of ischemic diseases [16]. In the recent years, the use of stem cell transplantation to promote neovascularization has achieved remarkable progress in the treatment of ischemic heart disease and peripheral arterial disease. Animal experiments and clinical trials have suggested that transplantation of EPCs can promote the formation of collateral circulation in ischemic limbs and myocardial infarction and accelerate reendothelialization after vascular injury [17, 18]. In 2005, for the first time, EPC was used to sperate from human umbilical vein blood to treat diabetic neuropathy of immunodeficient rats and achieved an exciting result [13].

The BM mesenchymal stem cells (MSCs) is an heatly reseached area until recently. In 1966, Friedenstein et. al. [19] firstly proposed BM MSCs as osteoblast progenitor cells. In the follow-up study, the transplantation of MSCs was found to improve ischemic diseases such as myocardial ischemia and atherosclerosis [20]. In 2008, Shibata et.al. [21] demonstrated for the first time that transplantation of BM MSCs can considerably improve the rat peripheral neuropathy: 4 weeks after the transplantation, local growth factors of bFGF and VEGF were expressively increased in the lower limb and the capillary of soleus muscle was improved significantly. Besides, the sciatic nerve blood flow perfusion (SNBF) also was enhanced with the enlarged nerve conduction velocity.

As the contents of EPCs and MSCs are low, the practical method mainly includes separation of EPCs and MSCs followed by amplification in vitro for transplantation. Although these two kinds of cells are the best candidate cells to promote angiogenesis theoretically, each one

has shortcomings in clinical application: EPCs and MSCs are of low content that require long time to culture to obtain enough EPCs and MSCs in vitro and during this period, the cells may be subjected to pollution or differentiation. Currently, in clinical use of BM or peripheral blood stem cell after mobilization, the application of autologous BM stem cell in transplantation has been widely applied.

This study took local multi-point injection of SDF-1α combined with BM transplantation, aimed at increasing the effect of BM transplantation. The experimental results suggested that for the improvement of sensitivity to pain, the conduction velocity of sciatic nerve and the synthesis and secretion function of sciatic nerve neurotrophic factor tended to be greater than that in BM transplantation alone, though without statistical difference. We think the following factors may be responsible for the obtained results. First, a study found that BM stem cells can play a localized role lasting more than 12 weeks, while SDF-1α has a finite life for its use and degradation in a localized setting. Second, SDF-1α is a protein prone to adhere to the syringe. Although we used a low concentration of BSA rinse syringes, SDF-1α may adhere partly. In addition, since the price of pure SDF-1α is expensive, the direct injection method has considerable economic challenges. To further investigate the research, we planned to construct SDF-1 eukaryotic expression vector to assure the stability SDF-1 expression in the diseased limb locally, and achieve sustained SDF-1α action.

Nerve fibers themselves secrete angioneurins that have dual roles of promoting angiogenesis and nourishing nerves. For example, VEGF and bFGF are important for maintaining the structure and function of the nerve fibers. This study showed that allogeneic progenitor cell transplantation can increase VEGF, bFGF and Gli in the sciatic nerve of rats [22], which was positively related with the improvement of sciatic nerve function. We speculated that, autologous bone marrow transplantation combined with SDF-1α on sciatic nerve function improvement may be associated with increased synthesis and secretion of angioneurins, in addition to local angiogenesis, increased blood supply, and improved micro environment. The results suggested that autologous bone marrow transplantation combined with SDF-1α can improve the effect of transcription of neurotrophic factors of the sciatic nerve in diabetic rats, and closely correlate with the improvement in structure and function.

In summary, autologous bone marrow transplantation combined with SDF-1α administered locally can effectively improve peripheral neuropathy of diabetic rats and provide a new strategy for the treatment of DNP. Further studies are warranted on this potent clinical application.

Disclosure of conflict of interest

None.

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