Int J Clin Exp Med 2016;9(2):2245-2252
www.ijcem.com /ISSN:1940-5901/IJCEM0014154

Original Article

Mechanism of the therapeutic effect of bone marrow-derived mesenchymal stem cell transplantation on limb ischemia in SD rats

Chao Bai[1*], Yang Wang[1*], Xinxi Li[1], Chenming Guo[2], Ye Tian[1], Wenwen Yang[3], Jun Luo[1]

Departments of [1]Thyroid Surgery, [2]Breast Surgery, The First Teaching Hospital of Xinjiang Medical University, Urumqi, Xinjiang, China; [3]Department of Endocrinology, The Second Affiliated Hospital of Xinjiang Medical University, Urumqi, Xinjiang, China. [*]Equal contributors.

Received August 8, 2015; Accepted November 19, 2015; Epub February 15, 2016; Published February 29, 2016

Abstract: Objective: The aim of this work was to explore the therapeutic effect of bone marrow-derived mesenchymal stem cells transplantation in SD rats' ischemic hind limb. Methods: Ligation of the abdominal aorta beneath the renal artery, the lumbar artery and the iliolumbar artery, was performed to obtain the hindlimb ischemia model in female SD rats. Amplified and purified bone marrow-derived mesenchymal stem cells from male SD rats were injected into the rectus femoris of the right hind limb of the ischemia rats, while the control group received an injection of an equal amount of saline. Two, four and six weeks after the injection, a sample of the right rectus femoris was collected for the immunohistochemical detection of the vascular endothelial growth factor and sex-determining region Y (SRY). Results: Both the capillary number on the right rectus muscle and the vascular endothelial growth factor positive cell in the transplant group were significantly higher compared to the control group at 2, 4 and 6 weeks after transplantation ($P < 0.01$). The SRY positive cells were detected in the capillary walls and scattered in the muscle tissue of the rectus femoris of the transplanted rats. Conclusion: Bone marrow-derived mesenchymal stem cell transplantation may increase the vascular endothelial growth factor level in ischemic tissues, participate in the new capillary formation in the ischemic limbs, promote rapid capillary growth in the ischemic tissue, and improve the blood supply to the ischemic hind limb.

Keywords: Bone marrow-derived mesenchymal stem cells, mechanism; limb ischemia, SD rats, vascular endothelial growth factor

Introduction

With the improved living quality and alternative lifestyle, the incidence of chronic ischemic disease of the lower extremities, such as lower extremity arterial occlusive disease, thromboangiitis obliterans, and diabetic ischemic lower extremity vascular disease, has gradually increased. Benefited from the constantly advancing medical technology, the diagnosis and treatment of the chronic lower limb ischemia disease have also been greatly improved [1-3]. However, the efficacy of the treatments is still less than ideal in patients with poor blood circulation at the lower extremity, especially in older patients, patients with underlying diseases and with severe occlusion of the distal lower extremities without collateral circulation. The disease progression could ultimately lead to amputation and eventually death.

In the late 1990s, the idea of treating the ischemic lower extremity by an angiogenic therapy was proposed and proved to be feasible by a large amount of basic research and animal experiments. Kawamura *et al.* [4] confirmed that cells derived from the $CD34^+$ bone marrow could differentiate *in vitro* into endothelial progenitor cells and induce the formation of the inner surface of the small blood vessels *in vivo*. In addition, the bone marrow stromal stem cells can secrete basic fibroblast growth factor and vascular prosthesis lumen endothelial factor to promote angiogenesis. In clinical practice, Tateishi-Yuyama *et al.* [5] were the first to report the application of autologous bone marrow

stem cell transplantation in the treatment of lower limb ischemia and set a precedent in the clinical application of angiogenesis. Bone marrow-derived mesenchymal stem cells isolated from bone marrow possess multilineage differentiation potential [6-8], and could differentiate into the three types of germ cells. Vascular endothelial cells, differentiated from bone marrow stem cells by artificially induction, can be used in the treatment of ischemic disease of lower extremity mediated by the formation of new blood vessels and in the improvement of the blood supply to the ischemic tissue.

In recent years, autologous stem cell transplantation has become a novel treatment of ischemic disease of lower extremity [9]. The transplantation of autologous bone marrow stem cells, which can promote the formation of new blood vessels, improve the ischemic limb blood flow and accelerate the healing of ulcers of the skin, bringing new hope for the treatment and prognosis of patients with chronic ischemic lower extremity disease. Currently, the mechanism of autologous stem cell transplantation in promoting neovascularization in ischemic limb, whether mediated by stimulation of the secretion of related cytokines to induce the growth factors gene expression or by regulation of the immune system and to maintain the cell membrane stability, has still been disputed. It is also possible that they are mediated by some mechanism still unknown. In this study, we induced the hindlimb ischemia model in SD rat and treated it with transplanted bone marrow-derived mesenchymal stem cells. Compared with the saline control group, we explored the effectiveness and the mechanism of bone marrow-derived mesenchymal stem cell transplantation in the treatment of limb ischemia by histological analysis, providing the basis for the clinical application in the treatment of limb ischemic diseases.

Materials and methods

Materials

Experimental animals: The experiments were conducted from June to December 2014 at the Laboratory Animal Center of the Xinjiang Medical University. Fifty SD rats from a closed colony, raised under specific-pathogen-free (SPF) conditions, were provided by the Laboratory Animal Center of the Xinjiang Medical University [production license number: SCXK (new) 2011-003; Usage permit number: SCXK (new) 2011-0001]. The animals use and the experimental setting were approved by the Hospital Ethics Committee (Approval number: New A-0120723004). Animals were housed in temperature, humidity and wind-controlled rooms at 20-25°C, 40%-70% and 0.1-0.2 m/s. respectively, under a cycle of 12 hours light and 12 hours dark. Two 4-week-old male SD rats with an average body weight of 146.5 g were used for cell culture. Forty-eight female SD rats (average age: 5.0 ± 0.6 weeks; average body weight: 156 ± 0.6 g) were used for the induction of the hind limb ischemia.

Reagents and apparatus: L-DMEM culture medium (Gibco, United States), special-grade 10% fetal bovine serum (Hyclone, United States), mouse anti-rat CD29, CD34, CD44, CD45 monoclonal antibodies (Sigma, United States), rabbit anti-rat SRY polyclonal antibody (Santa Cruz, United States), rabbit anti-rat vascular endothelial growth factor polyclonal antibody (Boshide, Wuhan, China), Leica DMI 4000 B optical microscopes and medical image analysis system (Leica, Germany), Leic 2135 microtome (Leica, Germany), Leica HI1210 water bath for paraffin section (Leica, Germany), HF240CO_2 incubator (Likang Biomedical technology Limited Co. Hong Kong), Jouan BR4 centrifuge (Jouan, France), SW-CJ-2FD two person one side clean workbench (Sujingantai Air Technology Limited Co. Suzhou, China), Flow cytometry (Becton-Dickinson, United States), OPMI pico p30020001 Surgical microscope (Carl Zeiss Meditec AG, Germany).

Experimental methods

Bone marrow-derived mesenchymal stem cells culture: The femurs and tibias from 2 adult male SD rats were soaked in PBS containing 100 U/mL penicillin and 100 U/mL streptomycin for 15 minutes, and then rinsed twice with PBS. The metaphysis was removed and the bone marrow was flushed out by syringe using 5 mL L-DMEM culture media containing 10% fetal bovine serum, 2 mmol/l l-glutamine, 100 U/mL penicillin and 100 U/mL streptomycin. Single bone marrow cell suspension was seeded in a plastic Petri dish at a concentration of 6-8 × 10^9/L. Half of the culture medium was changed after 48 hours of culture and subsequently every 3 days until the adherent cell

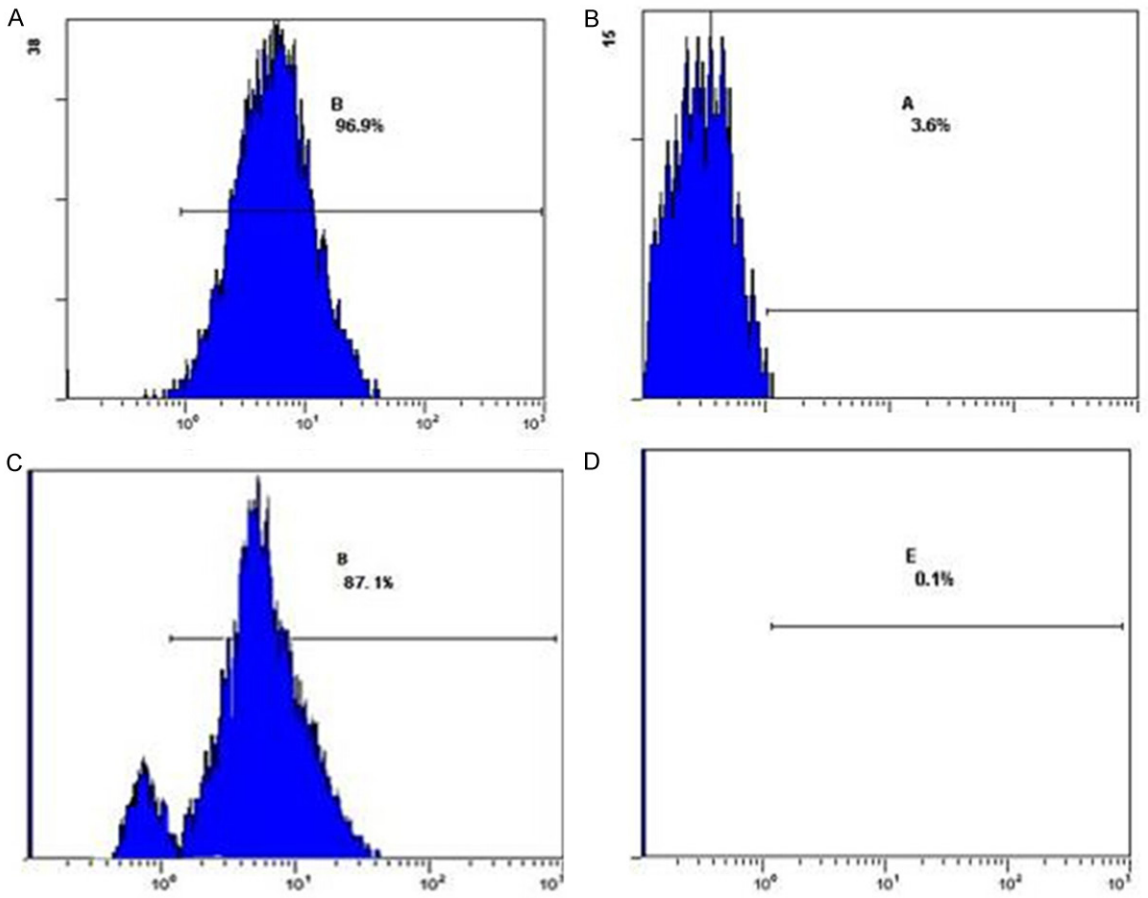

Figure 1. Flow cytometry detection of bone marrow-derived mesenchymal stem cell surface markers. Note: A: 96.9% CD29; B: 3.6% CD34; C: 87.1% CD44; D: 0.1% CD45.

reached 80%-90% confluence. The culture medium was discarded, and the cells were washed twice with PBS and digested by 0.25% trypsin solution. The cells were collected and subcultured at the concentration of 6-8 × 10^6/L. The fifth generation cells were used for transplantation and flow cytometry was used to check the expression of surface markers.

Animal groups: Forty-eight female SD rats were randomly divided into two groups: the transplant group and the control group, with 24 rats in each group.

The hind limb ischemia model induction and post-operation treatment: Rats were anesthetized by an intraperitoneally injection of ketamine solution at the concentration of 60 mg/kg. The rat was kept in a supine position with its limbs fixed with adhesive tape and the abdomen was disinfected by 0.5% iodophor application. A 3 cm median abdominal incision was made upward from the bilateral anterior superior iliac spine line plane. The intestine was pushed on the top-left and the rear peritoneum was cut to expose the abdominal aorta, the lumbar artery, and the iliolumbar artery. The abdominal aorta and the bilateral iliolumbar arteries below the renal artery were ligated using a 5-0 silk thread, the lumbar arteries were ligated using 11-0 silk thread and then the abdominal cavity was closed. The rat was able to drink water immediately after recovering from anesthesia, and was able to eat normally from the second day onward. Penicillin (2.0 × 10^6 U/kg) was injected intraperitoneally for 3 consecutive days and the stitches were removed 5 days after the operation.

Bone marrow-derived mesenchymal stem cell transplantation: Five days after the operation, 0.25 mL bone marrow-derived mesenchymal stem cell suspensions at a concentration of 1 × 10^6 cells were injected into 5 different locations on the right rectus femoris muscle of the experimental group rats. Another 0.25 mL

Table 1. Number of capillaries in the right rectus femoris muscle in the rats of each group ($\bar{x} \pm s$, $n = 8$)

Group	2 weeks post-transplantation	4 weeks post-transplantation	6 weeks post-transplantation
Control group	17.50 ± 4.57	12.63 ± 5.63	12.88 ± 7.81
Transplant group	50.00 ± 14.02	52.25 ± 10.38	46.13 ± 11.73
F	38.863	90.148	44.549
P	0.000	0.000	0.000

saline solution was injected into the same locations in the control group rats.

Main outcome measures: Eight rats from both the transplant group and the control group were sacrificed at two, four and six weeks after transplant, and the right rectus femoris muscles were removed and sectioned. The Hematoxylin-eosin staining was performed as follows: Hematoxylin stained for 10 minutes, washed, then returned to the blue after differentiated with hydrochloric ethanol; Next, Eosin stained for 15 minutes, washed, made transparent in 2 changes of xylene and sealed at last. A number of capillaries from 5 randomly selected fields from each specimen were counted under an optical microscope with a 400 × magnification, In order to evaluate the number of capillaries. The immunohistochemical staining of vascular endothelial growth factor was performed as follows. The tissue sections were baked, dewaxed and hydrolyzed and the endogenous peroxidase was removed. After antigen retrieval and serum block, the tissue section was incubated with the primary antibody, and subsequently incubated with the biotinylated secondary antibody. DAB was used to stain the protein and hematoxylin was used for counterstaining, then the tissue section was sealed. The number of vascular endothelial growth factor positive cells was counted from 5 randomly selected fields of each specimen under an optical microscope at 400 × magnification, in order to evaluate the number of vascular endothelial growth factor positive cells. The immunohistochemical staining of SRY was performed following the same procedure described above. The distribution of the SRY positive cells was observed under an optical microscope.

Statistical analysis

SPSS 17.0 software was used for the statistical analysis. The data were expressed as $\bar{x} \pm s$ (mean ± s.d.). Single-factor analysis was used for variance tests. $P < 0.05$ was considered statistically significant.

Results

The animal experiment was performed without any significant technical problems, and no sign of complications was detected.

Cells surface markers expression in the transplanted cells

The flow cytometry analysis showed that 96.9% of the cells were CD29 positive, 87.1% were CD44 positive, 3.6% were CD34 positive, and 0.1% were CD45 positive. The observed results were consistent with the typical characteristics of the bone marrow-derived mesenchymal stem cells (**Figure 1**).

Capillary number

Single factor analysis of variance test showed that the number of the capillaries in the right rectus femoris muscle of the transplant group was significantly higher compared with the number in the control group ($P < 0.01$) at 2, 4, and 6 weeks after transplant, indicating that the ischemia in the transplant rats' group was significantly improved compared with the control group (**Table 1**; **Figure 2**).

Vascular endothelial growth factor positive cell

Single factor analysis of variance showed that the number of the vascular endothelial growth factor positive cells in the right rectus femoris muscle of the transplant group were significantly higher compared with the number in the control group ($P < 0.01$) at 2, 4 and 6 weeks after transplantation. The transplant group was significantly improved compared with the control group (**Table 2**; **Figure 3**).

Distribution of SRY positive cells

Six weeks after transplantation, some capillary endothelial cells were positive in the transplant group and SRY positive cells appeared to be scattered or in clusters both in and between the muscle tissues. No SRY positive cells were observed in the control group (**Figure 4**).

Discussion

Limb ischemia caused by arterial disease is associated with a high amputation rate. Drug

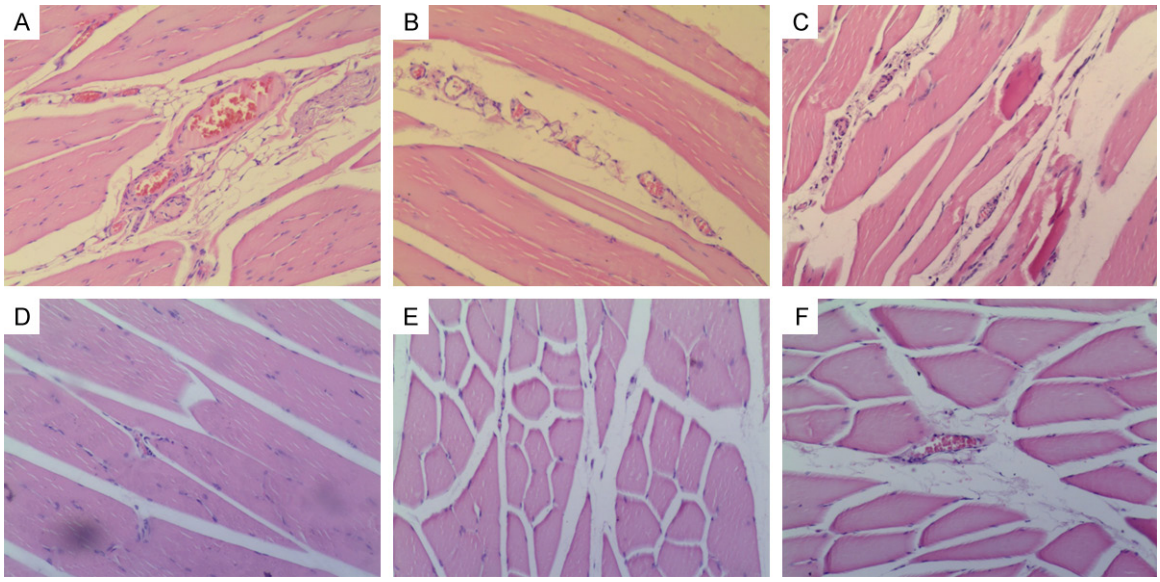

Figure 2. Hematoxylin-eosin staining of the right rectus femoris muscle of the rats of each group (× 400). Note: A-C: Transplant group right rectus femoris muscle at 2, 4 and 6 weeks after transplantation, respectively; D-F: Control group right rectus femoris muscle at 2, 4 and 6 weeks after transplantation, respectively.

Table 2. Vascular endothelial growth factor positive cells in the right rectus femoris muscle of the rats of each group

Group	2 weeks post-transplantation	4 weeks post-transplantation	6 weeks post-transplantation
Control group	14.63 ± 5.93	15.50 ± 5.10	15.88 ± 4.94
Transplant group	36.50 ± 2.45	36.38 ± 4.03	33.88 ± 3.00
F	93.085	82.477	77.621
P	0.000	0.000	0.000

therapy and surgical intervention can temporarily ease the patient's condition, but finally the amputation is needed. Stem cell transplantation has brought patients' hope. Currently, bone marrow stem cells are the most commonly used source of stem cells [10]. Bone marrow stem cells can be used to induce angiogenesis in the ischemic limb tissues, increase blood flow, reduce the pain and improve the limb salvage rates [11, 12]. In-depth study of the survival, tissue regeneration and repair mechanisms of bone marrow stem cells transplant is very important in the evaluation and application of bone marrow stem cell transplantation in the treatment of lower limb ischemia [13]. In this study, bone marrow-derived mesenchymal stem cells were transplanted for the treatment of SD rat hind limb ischemia and the results were compared with the saline control group. The effectiveness and the mechanism of bone marrow-derived mesenchymal stem cells transplantation for the treatment of limb ischemia was discussed on the basis of the histology results.

Our results showed that the number of capillaries in the muscles of the ischemic limbs was significantly increased after the transplantation of the bone marrow-derived mesenchymal stem cells. The number of capillaries further increased two weeks after the transplantation, indicating that transplanted bone marrow-derived mesenchymal stem cells can induce a rapid generation of a large number of capillaries in the ischemic environment.

Vascular endothelial growth factor represents a specific and efficient mitogen for vascular endothelial cells [14]. The main functions of the vascular endothelial growth factor are: 1). Induce the synthesis of a large amount of proteolytic enzymes in the endothelial cells and accelerate the blood vessel formation to promote angiogenesis [15, 16]. 2) Enhance vascular permeability [17]. 3) Maintain the normal state and the integrity of blood vessels. Transplanted bone marrow-derived mesenchymal stem cells should be injected directly in the ischemia site [18, 19] to increase the vascular endothelial growth factor level in the ischemic limb tissues [20, 21]. In return, the increased

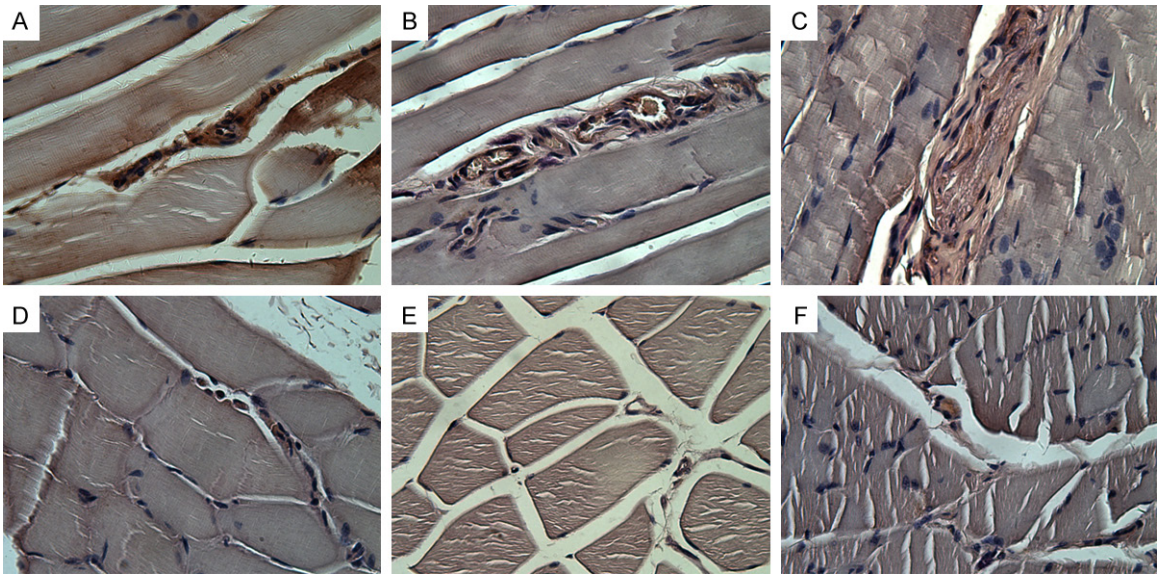

Figure 3. Vascular endothelial growth factor positive cell counting in each group by immunohistochemical staining (× 400). Note: A-C: Transplant group right rectus femoris muscle at 2, 4 and 6 weeks after transplantation, respectively; D-F: Control group right rectus femoris muscle at 2, 4 and 6 weeks after transplantation, respectively.

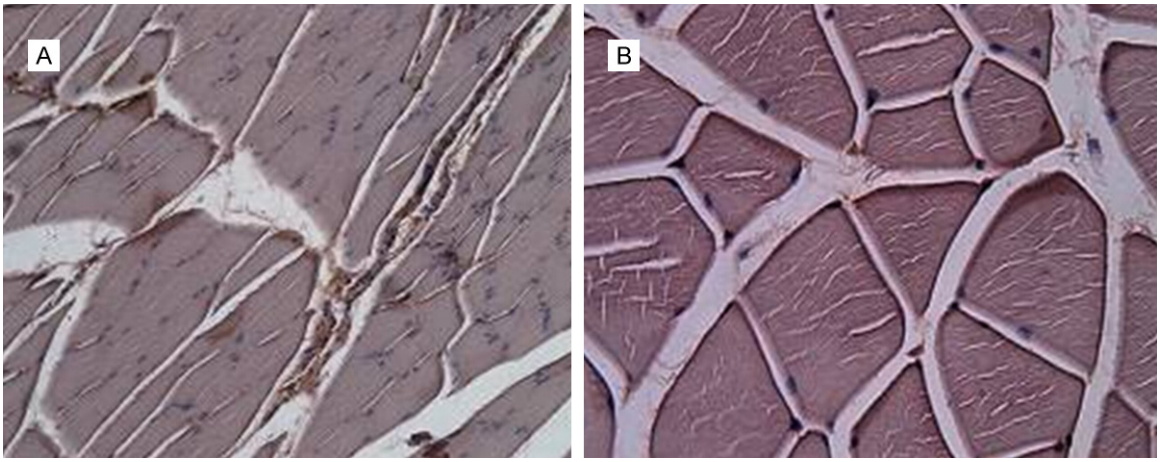

Figure 4. SRY positive cells distribution in the transplant and control groups 6 weeks after transplantation (× 400). Note: A: Transplant group; B: Control group.

vascular endothelial growth factor concentration can also increase the number of bone marrow-derived mesenchymal stem cells [22-24]. In this experiment, the number of vascular endothelial growth factor positive cells in the transplant group was remarkable increased compared to the control group, indicating that bone marrow-derived mesenchymal stem cell transplantation may significantly increase the vascular endothelial growth factor level in the ischemic tissues, although the source of the increased vascular endothelial growth factors could not be determined. We speculate that the vascular endothelial growth factor was secreted by the bone marrow-derived mesenchymal stem cells as a form of paracrine signal [25].

SRY is a male sex-determining gene which refers to the gene sequence on the Y chromosome that specifically defines male sex. In this experiment, bone marrow-derived mesenchymal stem cells from male SD rats were transplanted into female SD rats with ischemic lower extremities. The capillary endothelial cells in the transplanted SD female muscle were SRY positive, revealing that bone marrow-derived

mesenchymal stem cells may differentiate into vascular endothelial cells in ischemic limbs and participate in the formation of new capillaries in the ischemic tissue.

Conclusions

Our study has showed that transplanted bone marrow-derived mesenchymal stem cells induced a rapid generation of a large number of capillaries in the ischemic environment, and significantly increased vascular endothelial growth factor levels in ischemic tissues. It is surmised that bone marrow-derived mesenchymal stem cells differentiated into vascular endothelial cells in ischemic limbs, thus facilitating the formation of new capillaries in the ischemic tissue. Further studies are needed to explore the therapeutic potential of using bone marrow-derived mesenchymal stem cell transplantation in ischemic tissue.

Acknowledgements

This work was supported by grant (2010211-A48) from Natural Science Foundation of Xinjiang Uygur Autonomous Region. Ethical approval was given by the medical ethics committee of The First Teaching Hospital of Xinjiang Medical University.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Jun Luo, Department of Thyroid Surgery, The First Teaching Hospital of Xinjiang Medical University, Urumqi, Xinjiang, China. Tel: +86 13999218882; E-mail: 2369475216@qq.com

References

[1] Alavi A, Sibbald RG, Mayer D, Goodman L, Botros M, Armstrong DG, Woo K, Boeni T, Ayello EA, Kirsner RS. Diabetic foot ulcers: Part I. Pathophysiology and prevention. J Am Acad Dermatol 2014; 70: 1, e1-18.

[2] Schaper NC, Andros G, Apelqvist J, Bakker K, Lammer J, Lepantalo M, Mills JL, Reekers J, Shearman CP, Zierler RE, Hinchliffe RJ. Diagnosis and treatment of peripheral arterial disease in diabetic patients with a foot ulcer. A progress report of the International Working Group on the Diabetic Foot. Diabetes Metab Res Rev 2012; 28 Suppl 1: 218-224.

[3] O'Loughlin A, McIntosh C, Dinneen SF, O'Brien T. Review paper: basic concepts to novel therapies: a review of the diabetic foot. Int J Low Extrem Wounds 2010; 9: 90-102.

[4] Kawamura A, Horie T, Tsuda I, Kukita K, Yonekawa M. Regeneration of ischemic limbs by implantation of peripheral blood stem cells. Hokkaido Igaku Zasshi 2004; 79: 9-13.

[5] Tateishi-Yuyama E, Matsubara H, Murohara T, Ikeda U, Shintani S, Masaki H, Amano K, Kishimoto Y, Yoshimoto K, Akashi H, Shimada K, Iwasaka T, Imaizumi T. Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone-marrow cells: a pilot study and a randomised controlled trial. Lancet 2002; 60: 427-435.

[6] Hatzistergos KE, Quevedo H, Oskouei BN, Hu Q, Feigenbaum GS, Margitich IS, Mazhari R, Boyle AJ, Zambrano JP, Rodriguez JE, Dulce R, Pattany PM, Valdes D, Revilla C, Heldman AW, McNiece I, Hare JM. Bone marrow mesenchymal stem cells stimulate cardiac stem cell proliferation and differentiation. Circ Res 2010; 107: 13-22.

[7] Kassis I, Vaknin-Dembinsky A, Karussis D. Bone marrow mesenchymal stem cells: agents of immunomodulation and neuroprotection. Curr Stem Cell Res Ther 2011; 6: 63-68.

[8] Mohamadnejad M, Sohail MA, Watanabe A, Krause DS, Swenson ES, Mehal WZ. Adenosine inhibits chemotaxis and induces hepatocyte-specific genes in bone marrow mesenchymal stem cells. Hepatology 2010; 1: 963-973.

[9] Dhinsa BS, Adesida AB. Current clinical therapies for cartilage repair, their limitation and the role of stem cells. Curr Stem Cell Res Ther 2012; 7: 143-148.

[10] Bai K, Huang Y, Jia X, Fan Y, Wang W. Endothelium oriented differentiation of bone marrow mesenchymal stem cells under chemical and mechanical stimulations. J Biomech 2010; 43: 1176-1181.

[11] Schiavetta A, Maione C, Botti C, Marino G, Lillo S, Garrone A, Lanza L, Pagliari S, Silvestroni A, Signoriello G, Sica V, Cobellis G. A phase II trial of autologous transplantation of bone marrow stem cells for critical limb ischemia: results of the Naples and Pietra Ligure Evaluation of Stem Cells study. Stem Cells Transl Med 2012; 1: 572-578.

[12] Hamano K, Li TS, Kobayashi T, Tanaka N, Kobayashi S, Matsuzaki M, Esato K. The induction of angiogenesis by the implantation of autologous bone marrow cells: a novel and simple therapeutic method. Surgery 2001; 130: 44-54.

[13] Park JS, Hashi C, Li S. Culture of bone marrow mesenchymal stem cells on engineered matrix. Methods Mol Biol 2010; 621: 117-137.

[14] Bonnefond A, Saulnier PJ, Stathopoulou MG, Grarup N, Ndiaye NC, Roussel R, Nezhad MA, Dechaume A, Lantieri O, Hercberg S, Lauritzen T, Balkau B, El-Sayed Moustafa JS, Hansen T, Pedersen O, Froguel P, Charpentier G, Marre M, Hadjadj S, Visvikis-Siest S. What is the contribution of two genetic variants regulating VEGF levels to type 2 diabetes risk and to microvascular complications? PLoS One 2013; 8: e55921.

[15] Liang K, Jiang Z, Zhao B, Shen J, Huang D, Tao L. The expression of vascular endothelial growth factor in mast cells promotes the neovascularisation of human pterygia. Br J Ophthalmol 2012; 96: 1246-1251.

[16] Odemis V, Boosmann K, Heinen A, Küry P, Engele J. CXCR7 is an active component of SDF-1 signalling in astrocytes and Schwann cells. J Cell Sci 2010; 123: 1081-1088.

[17] Kwon MJ, An S, Choi S, Nam K, Jung HS, Yoon CS, Ko JH, Jun HJ, Kim TK, Jung SJ, Park JH, Lee Y, Park JS. Effective healing of diabetic skin wounds by using nonviral gene therapy based on minicircle vascular endothelial growth factor DNA and a cationic dendrimer. J Gene Med 2012; 14: 272-278.

[18] Xiong N, Zhang Z, Huang J, Chen C, Zhang Z, Jia M, Xiong J, Liu X, Wang F, Cao X, Liang Z, Sun S, Lin Z, Wang T. VEGF-expressing human umbilical cord mesenchymal stem cells, an improved therapy strategy for Parkinson's disease. Gene Ther 2011; 18: 394-402.

[19] Hollweck T, Marschmann M, Hartmann I, Akra B, Meiser B, Reichart B, Eblenkamp M, Wintermantel E, Eissner G. Comparative analysis of adherence, viability, proliferation and morphology of umbilical cord tissue-derived mesenchymal stem cells seeded on different titanium-coated expanded polytetrafluoroethylene scaffolds. Biomed Mater 2010; 5: 065004.

[20] Fan W, Crawford R, Xiao Y. The ratio of VEGF/PEDF expression in bone marrow mesenchymal stem cells regulates neovascularization. Differentiation 2011; 81: 181-191.

[21] Yuan H, Dong DN, Jin X, Zou YX, Wu XJ, Kong XQ, Zhang JY, Gao BB, Zhou H. Bone marrow mesenchymal stem cells combined with VEGF gene for the treatment of limb ischemia in rabbits. Chinese Journal of General Surgery 2012; 27: 44-47.

[22] Deng J, Zou ZM, Zhou TL, Su YP, Ai GP, Wang JP, Xu H, Dong SW. Bone marrow mesenchymal stem cells can be mobilized into peripheral blood by G-CSF in vivo and integrate into traumatically injured cerebral tissue. Neurol Sci 2011; 32: 641-651.

[23] Tang JM, Wang JN, Zhang L, Zheng F, Yang JY, Kong X, Guo LY, Chen L, Huang YZ, Wan Y, Chen SY. VEGF/SDF-1 promotes cardiac stem cell mobilization and myocardial repair in the infarcted heart. Cardiovasc Res 2011; 91: 402-411.

[24] Thibeault S, Rautureau Y, Oubaha M, Faubert D, Wilkes BC, Delisle C, Gratton JP. S-nitrosylation of beta-catenin by eNOS-derived NO promotes VEGF-induced endothelial cell permeability. Mol Cell 2010; 39: 468-476.

[25] Guiducci S, Manetti M, Romano E, Mazzanti B, Ceccarelli C, Dal Pozzo S, Milia AF, Bellando-Randone S, Fiori G, Conforti ML, Saccardi R, Ibba-Manneschi L, Matucci-Cerinic M. Bone marrow-derived mesenchymal stem cells from early diffuse systemic sclerosis exhibit a paracrine machinery and stimulate angiogenesis in vitro. Ann Rheum Dis 2011; 70: 2011-2021.