Neuroscience Letters xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research article

Human mesenchymal stem cells improve the neurodegeneration of femoral nerve in a diabetic foot ulceration rats

Nan Xia [1], Jin-Mei Xu [1], Nan Zhao, Qing-Song Zhao, Ming Li *, Zhi-Feng Cheng *

Department of Endocrinology and Metabolism, the Fourth Affiliated Hospital of Harbin Medical University, Harbin, China

HIGHLIGHTS

- hMSCs-UC partially reverse the degeneration of FN via upregulation of NGF, NF-200 and angiogenesis.
- hMSCs-UC reverse at least partially the damages in myelination of FN revealed by EM data.
- The function of FN is improved after by hMSCs-UC revealed by compound action potential recording.
- This finding supports hMSCs-UC as a potential strategy for treatment of diabetic neuropathy.

ARTICLE INFO

Article history:
Received 4 January 2015
Received in revised form 22 April 2015
Accepted 23 April 2015
Available online xxx

Keywords:
Human mesenchymal stem cells from umbilical cord matrix
Diabetic foot
Peripheral neuropathy
NGF
Neurofilament
Electroneurogram

ABSTRACT

Neuropathy is observed in 50% of diabetic patients with diabetic foot. This study attempted to explore the potential role of human mesenchymal stem cells-umbilical cord blood (hMSCs-UC) in femoral nerve (FN) neuropathy. The model rats were established by one time administration of streptozotocin and empyrosis on the dorsal hind foot. At 3d, 7d, 14d after treatment with hMSCs-UC or saline through left femoral artery, the serum NGF was examined by ELISA; NF-200 expression in FN was evaluated by immunohistochemistry; the diameter and roundness of FN, the ratio of capillary and muscular fiber of gastrocnemius were calculated under light microscope; and neuronal degenerations, such as demyelization, axonal atrophy, and loose arrangement of nerve fibers, were observed by electronic microscope. The results showed that, in hMSCs-UC-treated model rats, serum NGF was increased with higher positive rate of NF-200. Although the difference in FN diameters was not established among groups, improvement of roundness of FN was confirmed with increase in the numbers of capillary in FN-innervated gastrocnemius; additionally, degenerative neuropathy was significantly improved. Importantly, the functional study of electroneurogram (ENG) showed that, slowed conduction of FN in model rats was significantly restored by hMSCs-CU treatment. These data suggested that hMSCs-UC-treatment partially reverse the neuronal degeneration and nerve function of FN, which might be contributed by the upregulation of NGF with dramatic angiogenesis in FN-innervated gastrocnemius, consequently reversing neuronal structure and function, preventing or curing foot ulceration.

© 2015 Published by Elsevier Ireland Ltd.

1. Introduction

The increasing incidence of diabetes and its chronic complication earns widespread respect all over the world. The neuropathy, which might include painless damage, and infection due to diabetic foot ulceration, dramatically reduced the quality of life [1] in about 50% of diabetic patients [2]. Clinical evidence indicates that diabetic patients with simplified foot ulceration are rarely seen, whereas, the majority of them are already with neuropathic complication at the first time diagnosis. The effective therapeutic measures or managements could possibly reverse, at least partially, the neuro-degenerative changes at later stage of peripheral neuropathy [3]. Human mesenchymal stem cells-umbilical cord blood (hMSCs-UC), as multi-functional stem cells, demonstrates advanced property, such as low immunogenicity, rich source, and easy collection. Importantly, experiences in aspects of cell replacement therapies [4], tissue engineering [5], and gene therapy [6], have been accumulated. We have recently reported that hMSCs-UC specifically migrate to ulcerated tissue and stimulate the repair

* Corresponding authors at: Department of Endocrinology and Metabolism, the Fourth Affiliated Hospital of Harbin Medical University, #37 Yi-Yuan Street, Harbin 150001, China. Tel.: +86 189 0360 2198; fax: +86 451 8257 6770.
E-mail addresses: 1982795764@qq.com (M. Li), chengzhifeng@ems.hrbmu.edu.cn (Z.-F. Cheng).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.neulet.2015.04.038
0304-3940/© 2015 Published by Elsevier Ireland Ltd.

UNCORRECTED PROOF

Please cite this article in press as: N. Xia, et al., Human mesenchymal stem cells improve the neurodegeneration of femoral nerve in a diabetic foot ulceration rats, Neurosci. Lett. (2015), http://dx.doi.org/10.1016/j.neulet.2015.04.038

process by epithlialization via releasing cytokeratin and extracellular matrix formation [7]. Peripheral neuropathy is one of the key contributors to the development of diabetic foot ulcers [8], thus, to prevent or reverse the peripheral neuropathy in diabetic patients, it would prevent foot or help to recover from foot ulceration, which may in turn reduce the risk of lower extremity amputations. To answer this question, the effects of human mesenchymal stem cells on the peripheral neuropathy in a diabetic foot ulceration model rat were studied. These results demonstrated that the neurodegenerative alternations were significantly reversed in hMSCs-UC-treated model rats. The finding provides evidence for theoretical and potential clinical strategies in future treatment of diabetic foot ulcerations.

2. Methods

2.1. Experimental animals

Adult male Sprague–Dawley (SD) rats ($n = 120$, weighing 200 ± 15 g), were purchased from Chinese Shanghai SLAC Laboratory Animal Co., Ltd. (grade: clean, license: SCXK 2011-0005). All rats were maintained on a 12 h light cycle in the animal facility. Experimental protocols used in this experiment were approved by the Institutional Animal Care and Use Committee of the School of Medical Science, Harbin Medical University. All animal experiments were conducted in accordance with the "Principles of Laboratory Animal Care" (NIH publication no. 85-23, revised 1985; http://grants1.nih.gov/grants/olaw/references/phspol.htm), as well as with specific National Laws where applicable.

2.2. Reagent

Streptozotocin (STZ, Sigma, St. Louis, USA) was use to induce diabetes in the rat model. Protamine zinc 30R insulin (Wan-Bang Biochemical Medicine Co.. Ltd., Xuzhou, China) was used to maintain the blood glucose levels in diabetic rats. The hMSCs-UC was provided by Jing-Meng Stem Cell Tech. (Beijing, China). NF-200 mouse anti-mouse polycloned antibody, IgG-HRP sheep anti-mouse immunohistochemical test kit, and βELISA test kit for rat NGF were provided by Boster Biological Comp. (Wuhan, China). Histological/pathological analysis was carried out using imaging software (BA2000; Motic, Richmond, Canada). Other equipment utilized included a MyCyclerTM PCR (Bio-Rad Hercules, USA), a gel imaging analysis system (GIS-3000, Hema Medical Instruments, Guangdong, China), and a cell culture incubator (Binder, Tuttlingen, Germany).

2.3. Preparation and administration of hMSCs-UC

For the preparation and continuous cell culture of hMSCs-UC, and identification of particular biological properties of MSCs, the experimental protocols followed exactly the same procedures as previously described [7], and expended procedures were also provided (Supplemental materials). The hMSCs-UC were administrated through left femoral artery according to the methods [9]. The rats with diabetic foot ulcers were sacrificed at 3 days (3d), 7 days (7d), and 14 days (14d) after hMSCs-UC treatment and the part of FN and gastrocnemius were collected from the left dorsal hind foot for morphological and immunohistochemical analysis.

2.4. Model rats for diabetic foot ulceration

One week after one time intraperitoneal injection of 55 mg/kg of STZ, 92% of rats developed diabetes [10], with fasting blood glucose level over 16.7 mM [11]. To create a model for diabetic foot ulcers, we made a 4×4 mm empyrosis on the left side of dorsal hind foot of the diabetic rats at 10–12 weeks after treatment with STZ. This procedure could be repeated until the healing of ulceration was delayed for at least 14 weeks [7].

2.5. Serum NGF examined by ELISA

The rats were placed in an airtight induction chamber for inhalation of the volatile diethyl ether after the lower end of sternum was sterilized with 70% alcohol swab. Upon relaxation, the blood was collected directly by vertically inserting the needle into the heart along the left margin near the lower end of sternum and the serum was collected for detection the serum concentration of NGF using βELISA test kit.

2.6. Morphological evaluation under light microscope

FN and gastrocnemius from the left leg of different groups were fixed with 4% paraformaldehyde for 48 h, embedded in paraffin, and sectioned at 4.0 μm. After de-waxing twice for with dimethylbenzene, the tissue slices were dehydrated by successive concentrations of ethanol. Finally, the tissues were washed twice in distilled water. The tissues were then HE stained and the tissue slices were sealed rapidly with nature balsam and cover slips for microscopic analysis.

2.7. NF-200 determined by immunohistochemistry

~1.0 cm FN was collected and similar histological procedures were applied. After HE treatment, the tissues were immersed into 3% hydrogen peroxide for 10 min, treated with 0.3% citric acid for 10 min for necessary, and then the tissue slices were sealed for light microscope observation (Olympus-CH-CX21, Japan).

2.8. Specimen preparation for transmission electron microscopy

~5.0 mm FN segments were dissected and the specimen was prepared according to the protocols described previously [12]. The resin blocks containing the FN were thin sectioned using a diamond knife (Diatome; Electron Microscopy Sciences) at 70 nm, and sections were placed on either copper or nickel mesh grids. After drying on filter paper for a minimum of 1 h, the sections were stained with the heavy metals uranyl acetate and lead citrate for contrast. After drying, Digital images were acquired with electron microscope (H7650, Hitachi, Japan) for later analysis.

2.9. Electroneurogram (ENG)

This experiment was conducted following the protocols described in details previously [13]. Briefly, 1.0-V and 15-V stimulus intensities were used to determine the fast (myelinated fiber activation, >2.0 M/s) and slow (unmyelinated fiber activation, <2.0 M/s) components of compound action potential (CAP), respectively. The root-mean-square (RMS) value of the ENG was calculated for each epoch of data corresponding to ENG components in the compound nerve recording.

2.10. Data collection and statistical analysis

The average data were expressed as mean ± SD. Data were analyzed by the Wilcoxon rank-sum test, the Kruskal–Wallis H test, the chi-square test, and the t-test, where, appropriate using SAS v9.1 software. P values less than 0.05 was considered to be statistically significant.

UNCORRECTED PROOF

Please cite this article in press as: N. Xia, et al., Human mesenchymal stem cells improve the neurodegeneration of femoral nerve in a diabetic foot ulceration rats, Neurosci. Lett. (2015), http://dx.doi.org/10.1016/j.neulet.2015.04.038

Download English Version:

https://daneshyari.com/en/article/6280707

Download Persian Version:

https://daneshyari.com/article/6280707

Daneshyari.com