Neuroscience Letters 617 (2016) 150–155

Contents lists available at ScienceDirect

Neuroscience Letters

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

Research paper

Intravenous PEP-1-GDNF is protective after focal cerebral ischemia in rats

Yaning Liu[a], Shangwu Wang[c], Shijian Luo[a], Zhendong Li[a,*], Fengyin Liang[b], Yanan Zhu[c], Zhong Pei[b,*], Ruxun Huang[b]

[a] Department of Neurology, The Fifth Affiliated Hospital of Sun Yat-Sen University, Zhuhai 519000, China
[b] Department of Neurology, National Key Clinical Department and Key Discipline of Neurology, Guangdong Key Laboratory for Diagnosis and Treatment of Major Neurological Diseases, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou 510080, China
[c] Kai Nuo Bio. Sci. Co., Ltd., Guangzhou 510633, China

HIGHLIGHTS

- PEP-1-GDNF is neuroprotective by intravenous administration.
- PEP-1-GDNF reduces the infarct volume.
- PEP-1-GDNF promotes the neurogenesis in the hippocampus.
- PEP-1-GDNF improves the neurological function after stroke.

ARTICLE INFO

Article history:
Received 22 December 2015
Received in revised form 1 February 2016
Accepted 2 February 2016
Available online 11 February 2016

Keywords:
PEP-1-GDNF
Protein transduction domain
Infarct volume
Neurogenesis
Stroke

ABSTRACT

Glial cell line-derived neurotrophic factor (GDNF) is a potential therapeutic protein on a variety of central nervous system diseases including ischemic stroke. However, GDNF is a large molecule that cannot cross the blood–brain barrier (BBB), which is still intact in the early hours after stroke when neural rescue is possible. PEP-1 protein transduction domain can deliver protein cargo across the cell membrane and the BBB. In the present study, we generated a novel fusion protein PEP-1-GDNF and examined whether PEP-1-GDNF is protective in focal cerebral ischemia. PEP-1-GDNF (200 μg/kg) or PBS was intravenously applied over 5 min immediately after reperfusion of 90 min transient middle cerebral artery occlusion (MCAO). After 28 days, rats were deeply anesthetized and decapitated. Behavioral tests were performed during this period. The results showed that PEP-1-GDNF significantly reduced the infarct volume and improved behavioral function. Further, PEP-1-GDNF promoted the cell proliferation and differentiation in the dentate gyrus of the hippocampus and attenuated ischemia-induced learning and memory damage.

1. Introduction

Glial cell line-derived neurotrophic factor (GDNF) has potent neuroprotective effects in ischemic stroke via different delivery approaches [1]. For example, topical application of GDNF can decrease brain edema, attenuate apoptotic cells, and reduce the infarct volume after stroke [2,3]. In addition, elevated GDNF level by gene transfer can reduce the infarct size and improve behavioral function in a rat MCAO model [4]. Further, transplantation of neural stem and progenitor cells modified to secrete GDNF significantly increases the level of GDNF in the brain and promotes the migration and differentiation of the stem cells [5]. Stroke initiates damage to the brain and impairs neurological function; however, stroke also induces several endogenous protective mechanisms, such as neurogenesis, which in turn limits tissue damage and promotes plasticity [6]. Neurogenesis mainly occurs in the dentate gyrus (DG) of the hippocampus and the subventricular zone (SVZ) of lateral ventricles. Particularly, reports suggest that neurogenesis is linked with learning and memory processes in the hippocampus [7,8]. Interestingly, GDNF-family receptorα-1 (GFRα-1) is up-regulated in CA3 and the DG of the hippocampus after stroke, suggesting

Abbreviations: GDNF, glial cell line-derived neurotrophic factor; GFRα-1, GDNF-family receptorα-1; PTD, protein transduction domain; BBB, blood–brain barrier; DG, dentate gyrus; SVZ, subventricular zone; MCAO, middle cerebral artery occlusion; CCA, common carotid artery; ICA, internal carotid artery; ECA, external carotid artery; PBS, phosphate buffered saline; BrdU, 5-bromo-2deoxyuridine.

* Corresponding authors.
E-mail addresses: lizdsysu@163.com, ZDL809@yahoo.com (Z. Li), peizhong@mail.sysu.edu.cn (Z. Pei).

http://dx.doi.org/10.1016/j.neulet.2016.02.017
0304-3940/

that GDNF has an important role in the neurogenesis following stroke [9]. Indeed, GDNF has been reported to promote the cellular proliferation, survival, and differentiation of progenitor cells in the hippocampus [10,11]. Although GDNF has potent neuroprotective effects, it is limited for clinical application because of a lack of an efficient and noninvasive method to delivery GDNF across the blood–brain barrier (BBB). To overcome this problem, GDNF has been linked to the protein transduction domain (PTD) as a fusion protein, which enables it to cross cell membranes and the BBB [12]. PTDs are small peptides (typically 5–25 amino acids), which are used to deliver the normally impermeable cargo across the cell membrane and the BBB through systemic administration. Some PTDs have been used in experiments or clinical trials [13]. PEP-1, a kind of PTDs, has successfully delivered multiple nonpenetrating therapeutic proteins into the brain by intravenous administration [14–17].

In the present study, we generated a novel fusion protein PEP-1-GDNF and investigated whether intravenous PEP-1-GDNF was protective after ischemic stroke in rats. The results revealed that intravenous administration of PEP-1-GDNF at the starting of reperfusion was neuroprotective. The PEP-1-GDNF group displayed significantly smaller infarct volume compared with the PBS control group. In addition, PEP-1-GDNF-treated rats performed significantly better in the cylinder test and Morris water maze. Further, PEP-1-GDNF promoted the cell proliferation and differentiation in the hippocampus of ischemic brain.

2. Materials and methods

2.1. Expression and purification of recombinant PEP-1-GDNF fusion protein

The PEP-1-GDNF coding sequence was polymerase chain reaction amplified by use of primers: 5′-tcgagctcaggaggaaacgccatggagaaagaaacctggtgggaaacctggtggaccg-3′ and 5′-cctggtggaccgaatggtctcagccgaaaaaaaaacgtaaagtgtcaccagataaacaaa-tgg-3′ for 5′-terminue, and primer 5′-tcaagcttcagatacatccacaccttt-3′ for 3′-terminue (96 °C for 3 min, 97 °C for 30 s, 59 °C for 30 s, and 72 °C for 31 s for 35 cycles; 72 °C for 7 min). Purified polymerase chain reaction fragments were cloned into the Not1/Bamh1 sites of the pPET-45b vector. PEP-1-GDNF was expressed in *Escherichia coli* strain BL21 (DE3) pLysS (Novagen) and lysed by sonication. The *E. coli* lysate was denatured in 8 mol/L urea; the bacterial debris was pelleted; and the supernatant was subjected to metal-affinity chromatography using a Ni-NTA matrix (Qiagen). Salts were removed by gel filtration on Sephadex G-25 (Amersham Pharmacia Biotech). The identity of the protein was confirmed by Western blotting, and the protein concentration was estimated with the Bradford method, using bovine serum albumin (BSA, 2 mg/ml) as a standard.

2.2. Animals and surgery

Adult male Sprague-Dawley rats weighing 250–280 g were purchased from the Center for Experimental Animals of Guangzhou University of Chinese Medicine (Guangzhou, China) and maintained at a specific pathogen-free housing facility. All procedures performed on these animals were approved by the Institutional Animal Ethical Committee at Sun Yat-Sen University and were in accordance with the Guidelines for the Care and Use of Laboratory Animals of the National Institute of Health (Publication No. 80-23, revised 1996).

Transient middle cerebral artery occlusion (MCAO) was induced by the intraluminal filament technique, as described in our previous report [18]. Briefly, under anesthesia with chloral hydrate (350 mg/kg, i.p.), a midline neck incision was made, and the right common carotid artery (CCA), the internal carotid artery (ICA), and the external carotid artery (ECA) were exposed. After ligation of the ECA and the proximal CCA, an MCAO monofilament was gently inserted into the ICA through the CCA until it blocked the bifurcating origin of the middle cerebral artery (MCA). The filament was withdrawn, initiating reperfusion after 90 min of MCA occlusion.

2.3. Treatment

Immediately after reperfusion, rats were randomly divided into two groups: the PEP-1-GDNF treatment group ($N = 10$) and the PBS control group ($N = 8$). PEP-1-GDNF (200 μg/kg) was slowly injected via tail vein within 5 min according to the previous report [19]. The control group received equal volume of PBS. To evaluate the cell proliferation, all rats received BrdU (50 mg/kg, i.p. daily) for successive 2 weeks starting 24 h after reperfusion.

2.4. Cylinder test

The cylinder test was performed at 7, 14, 21, and 28 days after drug treatment to assess asymmetric forelimb use. Briefly, the rats were placed inside a clear glass cylinder (Ø 20 cm). In total, 20 wall placements of the right forelimb (R), left forelimb (L), and both forelimbs simultaneously (S) were recorded [20]. Finally, the parameter was calculated as Limb Use Asymmetry Score = (R−L)/(R + L + S) [21].

2.5. Morris water maze

This test was chosen to evaluate spatial learning and memory of rats after reperfusion according to previous reports [22,23]. Briefly, from 21 to 27 days after stroke, rats were allowed to swim in a 1.3-m diameter pool filled with water for 60 s to search for the platform submerged under 1.5 cm of the water surface. Rats that located the platform were allowed to remain on it for 10 s, and rats that did not reach the platform during 60 s were gently guided to the platform and located on it for 10 s. The mean time that rats spent in the water during three attempts each day was collected to investigate the spatial learning. The probe trial was performed for each animal at day 28 of the experiment. In this trial, the platform was removed, and each animal was allowed to search for the platform for 60 s in the water at a predetermined location. Time spent in the goal quadrant was calculated to evaluate how well rats remembered the location of the platform.

2.6. Infarct volume calculation

At 28 days after surgery, all rats were sacrificed under deep anesthesia. The rats were transcardially perfused with saline, followed by 4% paraformaldehyde. Five coronal brain sections of 20 um thickness with equal space (2 mm apart, between −5.80 and +2.20 mm from Bregma; based on the Rat Brain in Stereotaxic Coordinates of Paxinos and Watson 2005) were prepared. Then the sections were stained with hematoxylin and eosin (HE), and the infarct volume was calculated as a percentage of the area of the contralateral hemisphere, and multiplied by the distance between sections to obtain the respective volume as reported previously [24,25].

2.7. Immunohistochemistry

Fluorescence double staining was used for visualization of BrdU and Nestin, GFAP, or NeuN, according to the protocol reported elsewhere [26]. In brief, series of every fifth section (150 μm apart) through each hippocampus were processed. Sections were incubated in 2 N HCl for 30 min at 37 °C and rinsed in 0.1 M borate