Neuroscience Letters 611 (2016) 14–20

Contents lists available at ScienceDirect

Neuroscience Letters

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

Research paper

Intraventricular infusion of a low fraction of serum enhances neurogenesis and improves recovery in a rodent stroke model

Yi-Chen Li [a], Li-Kai Tsai [b,**], Tai-Horng Young [a,*]

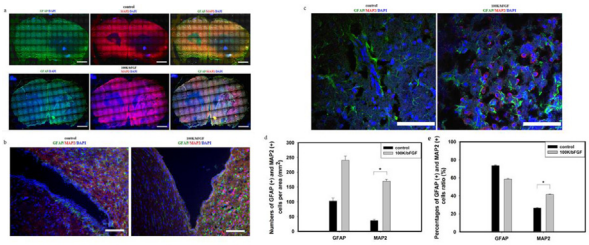
[a] Institute of Biomedical Engineering, College of Medicine and College of Engineering, National Taiwan University, Taipei 100, Taiwan
[b] Department of Neurology and Stroke Center, National Taiwan University Hospital and College of Medicine, Taipei 100, Taiwan

HIGHLIGHTS

- A serum fraction (100K) was easily derived from serum.
- 100K/bFGF enhanced cell proliferation at SVZ area and infarcted brain.
- 100K/bFGF increased the number of MAP-2 cells at infarcted brain in MCAO rat.
- 100K/bFGF improved animals' motor coordination of MCAO rat.

GRAPHICAL ABSTRACT

The cellular distribution of ischemia rat with or without the treatment of 100 K/bFGF.



The cellular distribution of ischemia rat with or without the treatment of 100K/bFGF.

ARTICLE INFO

Article history:
Received 15 June 2015
Received in revised form
27 September 2015
Accepted 12 November 2015
Available online 17 November 2015

Keywords:
Middle cerebral artery occlusion
Neurogenesis
Serum
Stem cell therapy
Stroke

ABSTRACT

Enhancing endogenous neurogenesis is a potential therapeutic strategy in stroke treatment. We have previously demonstrated that treatment with a fraction of serum with molecular weight of less than 100 kDa (100K) combined with bFGF promoted neurogenesis of cultured stem and progenitor cells (NSPCs). In this study, we further evaluated the efficacy of intraventricular administration of 100K with bFGF (100K/bFGF) in a rat model of transient middle cerebral artery occlusion (MCAO). Rats administered 100K/bFGF on post-stroke day 1 exhibited a higher number of Ki67 and Nestin immunoreactive cells at the subventricular zone (SVZ) area and in the infarcted brain, indicating promotion of NSPCs proliferation. The 100K/bFGF treatment also predominantly increased the number of MAP-2 immunoreactive cells rather than GFAP immunoreactive cells at the SVZ area and in the infarcted regions, implying that 100K/bFGF dominated NSPCs differentiating into neurons rather than astrocytes. Importantly, treatment with 100K/bFGF significantly improved the animals' motor coordination. These findings demonstrated that treatment with a low serum fraction and bFGF benefited ischemic stroke likely through promotion of the proliferation and neuronal differentiation of endogenous NSPCs.

1. Introduction

Stroke is one of the major causes of death and disability all over the world [3]. Since thrombolytic therapy only benefits a small group of stroke patients, recent extensive investigations for stroke are also focusing on neuroprotective treatment and regenerative therapies [15,32]. Adult neural stem and progenitor cells (NSPCs) locate at the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone of the hippocampal dentate gyrus, where they give rise to neurons throughout adulthood [6,33]. Adult neurogenesis is occurred in all mammalian species and may serve to replace cells damaged by brain insults [14]. Focal cerebral ischemia stimulates NSPC proliferation at SVZ and induces NSPC migration toward infarct regions, followed by neurogenesis [10,20]. Con-

* Corresponding author. Fax: +886 2 23940049.
** Corresponding author. Fax: +886 2 2341-8395.
E-mail addresses: milikai@ntuh.gov.tw (L.-K. Tsai), thyoung@ntu.edu.tw (T.-H. Young).

http://dx.doi.org/10.1016/j.neulet.2015.11.020
0304-3940/

ditional ablation of NSPCs in adult mice diminished poststroke motor and cognitive functional improvement and reduced synaptic connectivity [11,12,24], which demonstrated the importance of endogenous neurogenesis in stroke recovery. Therefore, enhancing endogenous neurogenesis may be a potential therapeutic strategy in stroke treatment.

Several endogenous growth factors are able to promote neurogenesis after ischemia neurogenesis is observed [13]. Neurotrophic factors, such as brain-derived neurotrophic factor (BNDF) [5] and insulin-like growth factor (IGF-1) [34], both enhanced cell proliferation in SVZ and facilitated angiogenesis in infarcted areas, paralleled by function improvement in rodent stroke models. Hematopoietic growth factors, including granulocyte-colony stimulating factor (G-CSF) [7] and erythropoietin [29], were able to trigger neurogenesis, suppress inflammatory reactions, and reduced stroke volume after rodent cerebral ischemia. Since these growth factors are normally present in blood stream, blood serum thus contains various beneficial elements for stroke therapy.

It is well-known that blood serum could support the survival, enhance proliferation, and promote differentiation of numerous cell types *in vitro* [19,25,28]. However, cultured NSPCs treated with serum differentiated predominantly into astrocytes rather than neurons [2,9,22], impeding further application in the disease therapy. It may be because the differentiation of NSPCs could be influenced by numerous factors in serum, including growth factors, cytokines, neurotransmitters, and electrolytes, etc [16]. Nevertheless, we recently demonstrated that treatment using only a fraction of serum with molecular weight of less than 100 kDa (100K) combined with basic fibroblast growth factor (bFGF) dominated the differentiation of NSPCs into neurons [18]. Here we try to apply this treatment strategy, treatment of 100K with bFGF (100K/bFGF), in stroke therapy. This study is aimed to investigate whether continuous intraventricular infusion of 100K/bFGF would enhance the neurogenesis of endogenous NSPCs and improve the functional recovery in an experimental model of transient cerebral ischemia.

2. Materials and methods

2.1. Serum fraction

The serum fraction was obtained and collected according to protocols described previously [18]. Serum fractions with molecular weight less than 100 kDa (100K) were prepared by centrifugation of FBS (Biological Industries) with a molecular exclusion of 100 kDa (Amicon® Ultra, Millipore) at 2600 g at room temperatures. After collection of a low fraction of serum (100K), double-deionized water was added to the original volume.

2.2. Animal surgery

The animal model was following with the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health and approved from the Laboratory Animal Center at National Taiwan University College of Medicine (Taiwan). The animal experiments for middle cerebral artery occlusion (MCAO) were followed the protocols as described previously [27,30]. Briefly, male Wistar rats (270–350 g) were anesthetized with isoflurane (1.5% in a mixture of 70% N_2O and 30% O_2). A 4–0 nylon suture (Dermalon™) with a heat-produced round tip was inserted from the right common carotid artery into the right internal carotid artery and then to the Willis' Circle to occlude the origin of the right middle cerebral artery. During and after surgeries, the body temperature was maintained at 37 ± 1 °C throughout the surgery with a heating pad. Then, an hour after MCAO, the nylon suture was withdrawn for cerebral reperfusion. Supplemental Fig. 1 showed the cerebral infarcted areas using 2,3,5-triphenyltetrazolium chloride staining.

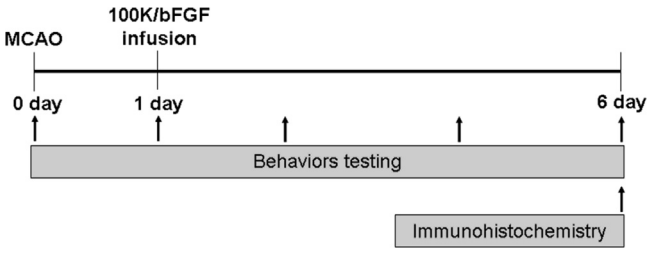


Fig. 1. Schematic representation of the time course of experimental design.

In the 100K/bFGF treatment group, an osmotic pump (Alzet®), containing whole 100K with bFGF at 20 ng/ml (Invitrogen), was placed subcutaneously on the back of each rat one day after MCAO. The 100K/bFGF solution was thus continuously infused for the following 5 days [17,21] into the right lateral ventricle of rats at stereotaxic coordinates: 1.5 mm lateral, 0.8 mm posterior to the bregma, and 3.5 mm below the brain surface. The time course of the study design was shown in Fig. 1. To validate the procedure of intraventricular infusion, we inoculated trypan blue solution into the right lateral ventricle of rats via the above infusion pump. The blue coloration was detected in the lateral and third ventricles, indicating the feasibility of the infusion technique (Supplemental Fig. 2).

2.3. Immunohistochemistry

The detailed methods for immunohistochemistry were described previously [18,27]. Briefly, animals were anesthetized with a lethal dose of isoflurane and fixed by transcardial perfusion with saline, followed by 4% formaldehyde. The brains were removed and submerged in 30% sucrose. A series of 20 μm-thick sections were then cyocut and stored at −80 °C. The brain sections were further incubated with primary antibodies, including anti-MAP (Millipore), anti-GFAP (Millipore), anti-nestin (Millipore), and anti-Ki67 (abcam) for 2 h at 37 °C. Samples were washed and incubated for 1.5 h at room temperature with the appropriate fluorescence-conjugated secondary antibodies. Samples were also counterstained with DAPI. These immunostained samples were then visualized by the fluorescent microscope (Lieca DMI600) and confocal microscope (Leica TPC SP5), under similar imaging conditions (brightness, contrast and intensity).

To quantify the cellular density in brain sections, we calculated the number of GFAP, MAP2, nestin, or Ki67 immunoreactive cells among the DAPI-stained cells in three low-power microscopic fields was evaluated in a randomized fashion. The average percentages of immunoreactive ratio within cells immunoreactive for anti-GFAP, MAP2 nestin, or Ki67 were calculated from the numbers of phenotypic marker-cells divided by the number of DAPI-stained cells. At least 200 cells were counted in each condition by a randomized fashion. Data were collected from three independent experiments for each condition.

2.4. Behaviors testing

Three behavioral tests (rotarod test, neurological severity score test, and body asymmetry test) were used to evaluate the motor function of rats before and after MCAO (Fig. 1) as our previous reports [27,30]. Detailed procedures are available online (Supplemental methods).