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Intranasal cerebrolysin improves cognitive function and structural synaptic plasticity in photothrombotic mouse model of medial prefrontal cortex ischemia

Saeed Sadigh-Eteghad[a], Mohammad Hossein Geranmayeh[a,b], Alireza Majdi[a], Farzad Salehpour[a], Javad Mahmoudi[a], Mehdi Farhoudi[a,*]

[a] Neurosciences Research Center, Tabriz University of Medical sciences, Tabriz, Iran
[b] Research Center for Pharmaceutical Nanotechnology, Tabriz University of Medical Science, Tabriz, Iran

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ABSTRACT

Medial prefrontal cortex (mPFC) ischemia affects post-stroke cognitive outcomes. We aimed to investigate the effects of different doses and routes of cerebrolysin (CBL) on the structural synaptic plasticity and cognitive function after mPFC ischemia in mice. Thence, CBL (1, 2.5 ml/kg/i.p./daily) or (1 ml/kg/i.n./daily), were administrated in photothrombotic mouse model of mPFC ischemia for two weeks. Episodic and spatial memories were assessed by the What-Where-Which (WWWhich) and Barnes tasks. Growth-associated protein 43 (GAP-43), postsynaptic density-95 (PSD-95), and synaptophysin (SYN) levels were measured in the lesioned area using western blot analysis. Dendritic arbors, spine densities, and morphology were assessed via Golgi-Cox staining. Treatment with 2.5 ml/kg/i.p. and 1 ml/kg/i.n. doses attenuated mPFC ischemia-induced episodic and spatial memories impairment. Results showed an obvious increase in the GAP-43, PSD-95 and SYN levels and improvement in the structural synaptic indexes in lesioned area induced by the same doses and routes of CBL. In conclusion, we found that specific doses/routes of CBL have positive effects on the structural synaptic plasticity and cognitive outcomes after mPFC ischemia.

1. Introduction

Ischemic stroke is the leading cause of brain dysfunction, resulting in sensory, motor, and cognitive impairment. However, no effective treatment has been yet introduced for restoring normal functions after stroke (Lu et al., 2017; Wang et al., 2017a; Zhang et al., 2017). Ischemic stroke in different cortical regions can impair learning and memory depending on the ischemia size and region (Schouten et al., 2009; Engstad et al., 2003).

Evidence suggests that medial prefrontal cortex (mPFC) plays a crucial role in episodic memory by its cooperation with other regions of the brain in acquiring, processing, storing, and retrieval of the information (Morici et al., 2015). It has also been shown that ischemic stroke in mPFC can impair spatial memory process initiation (Zhou et al., 2016). mPFC is required at different memory stages when the task cannot be solved by a single item strategy in rodents (Morici et al., 2015). It has been demonstrated that ischemic lesions in mPFC cause specific cognitive deficits (Deziel et al., 2015) and memory impairment in rodents (Zhou et al., 2016).

Photothrombotic model of stroke is beneficial to achieve highly reproducible cortical infarcts in rodents using which, the behavioral, cellular and molecular properties, can be delicately assessed (Labat-gest and Tomasi, 2013; Lee et al., 2007).

Post-stroke behavioral deficits can be attributed to the destruction of synapses and functional neuronal compartments such as axons and dendrites. On the other hand, structural synaptic plasticity is defined by spine number, size, and morphological alterations (Fu and Ip, 2017) all of which support long-lasting behavioral modifications (San Martin et al., 2017). Thus, assessment of dendritic spines density as an indicator of synaptic connections can be an appropriate tool for evaluating recovery process (Jeanneret and Yepes, 2016; Pei et al., 2015) as synaptic connections recovery results in functional improvement after stroke (Wu et al., 2014).

Detecting synaptic proteins levels such as growth associated protein 43 (GAP-43), postsynaptic density-95 (PSD-95), and synaptophysin (SYN) is helpful to show the regeneration after injury. GAP-43, as a marker of axonal growth, synaptogenesis and regeneration (Nemes et al., 2017; Jiang et al., 2017), PSD-95, as an essential factor in

* Corresponding author at: Neurosciences Research Center (NSRC), Tabriz University of Medical Sciences, 5166614756 Tabriz, Iran.
E-mail addresses: farhoudi_m@yahoo.com, farhoudim@tbzmed.ac.ir (M. Farhoudi).

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synaptic plasticity and postsynaptic membrane stabilizing (Luo et al., 2013; Vallejo et al., 2017), and SYN, as a marker of synapse development and activity (Yong et al., 2014; Dan et al., 2008) are the main proteins participating in the structural synaptic plasticity.

Cerebrolysin (CBL) is a complex lipid-free mixture of peptides and amino acids which act as neurotrophic factors (Kumaran Menon et al., 2012). Previous studies have shown that CBL administration after the acute phase of stroke improves neurological outcomes (Zhang et al., 2016; Zhang et al., 2010; Zhang et al., 2013; Ren et al., 2007), neurogenesis (Zhang et al., 2010; Zhang et al., 2013), white matter remodeling (Zhang et al., 2013), and neuronal tolerance to ischemic damage (Onishchenko et al., 2008). Hence, deciphering molecular, cellular, and behavioral effects of CBL on the brain afflicted by ischemic stroke helps understand its benefits and risks.

Intranasal (i.n.) route of drug delivery is a rapid, non-invasive and efficient method of brain drug targeting (Pourmemar et al., 2017) which has been approved in a variety of experimental and clinical studies (Domes et al., 2010; Craft et al., 2012; Farzampour et al., 2016). However, there is limited information about the effects of CBL on mPFC ischemia, its possibility, indications, and underlying mechanisms when administered via i.n. vs i.p. routes. In this study, we aimed to investigate the effects of different doses and administration routes of CBL on the structural synaptic plasticity and cognitive function after mPFC ischemia in mice.

2. Experimental procedure

2.1. Animals

In this study, 60 adult male BALB/c mice aged eight weeks and weighing between 25 and 30 g were used. Animals were housed in standard cages, 12/12 h light/dark cycle at 23 ± 2 °C temperature, with ad libitum access to food. All of the procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institute of Health (NIH; Publication No. 85–23, revised 1985). This study was ethically approved by National Institute for Medical Science Development (NIMAD) (No: IR.NIMAD.REC.1396.250).

2.2. Experimental design

After one-week of habituation of animals to their new environment, they were prepared for photothrombotic stroke induction and CBL administration. Thus the animals were randomly divided into 5 groups (n = 12 each): (I) the first group was considered as the sham surgery group, and other four groups were subjected to photothrombotic ischemia in mPFC area in both hemispheres, and subsequently received 14 days of treatment as follows; (II) normal saline (NS), (III) 1 ml/kg CBL (215.2 mg/ml, EVER Neuro Pharma GmbH, Unterach, Austria) via i.n. route (CBL 1 i.n.), (IV): 1 ml/kg CBL via intraperitoneal route (CBL 1 i.p.), (V): 2.5 ml/kg CBL via i.p. route (CBL 2.5 i.p.). All of the animals were included in the behavioral tests and randomly selected for molecular or histological testes. An 24 h "washing period" considered between behavioral testes. Fig. 1 shows the study design including the procedures and time scales.

CBL at 5 ml/kg/i.p. dose increased locomotor activity in the preliminary behavioral evaluations, so we did not include it in the study groups. For i.n. administration, drops containing 5–6 µl of CBL were administered nasally with alternation between right and left nares every two minutes up to 30 µl, depends on animal weight (Pourmemar et al., 2017).

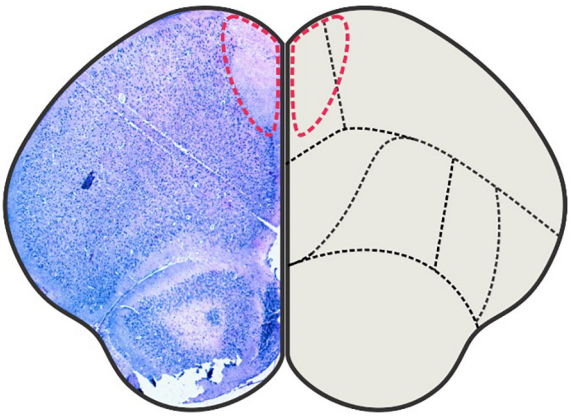

Fig. 2. Red dash shows the distribution of infarction site on medial prefrontal cortex (mPFC) region of mouse brain (Nissl staining) and drawings of the corresponding coronal section obtained from the Paxinos and Franklin atlas (Paxinos and Franklin, 2004). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.3. Photothrombotic ischemia model

Focal cerebral ischemia was simultaneously induced in both medial cortical regions. Briefly, after anesthesia induction by 2% isoflurane, animals were fixed in the stereotaxic frame, then the scalp was removed, and the laser was illuminated for 15 min (wavelengths = 532 nm at 17 mW, beam diameter = 2 mm) to the target area (centered approximately 2.2 mm anterior to the bregma). Rose Bengal (150 µg/g/i.p.) (Sigma-Aldrich, St. Louis, Missouri) was injected 5 min before starting illumination. During surgery procedure, the body temperature was kept at 37 °C. Afterwards, the wound was sutured, and animals were transferred into the recovery cages (Labat-gest and Tomasi, 2013; Lee et al., 2007). Ischemia induction approved by the Nissl staining, 48 h after surgery (Fig. 2).

2.4. Behavioral tests

2.4.1. What-where-which (WWWhich) test

WWWhich test was used to assess episodic-like memory using the method applied in Salehpour et al. study (Salehpour et al., 2017). The apparatus is comprised of two Plexiglas boxes (30 × 30 × 25 cm) which provide two different contexts. In context one, the floor was attached to a LEGO base plate, and the walls were painted matte black. While context two consisted of a smooth matte black floor and walls were painted in black and white vertical stripes. WWWhich test includes habituation, exposure, and test steps. On the first day, after five min of habituation in one of the contexts, two different objects were located in the same context and animals explored it for three min (exposure step). Subsequently, animals were transferred to the other context with the reverse arrangement of objects and different surface covers and maintained there for three min. After five min interval, the

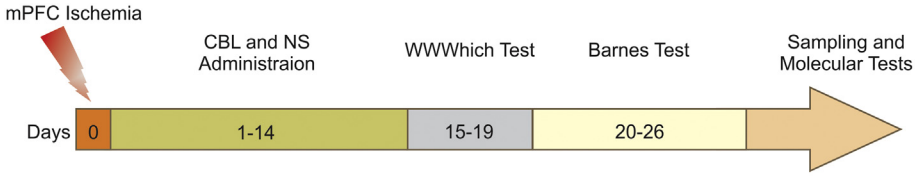

Fig. 1. Timescale of the medial prefrontal cortex (mPFC) ischemia induction, treatments administration, behavioral tasks, sampling and molecular tests.