Neuropharmacology 77 (2014) 453–464

Contents lists available at ScienceDirect

Neuropharmacology

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

Recombinant human thioredoxin-1 promotes neurogenesis and facilitates cognitive recovery following cerebral ischemia in mice☆

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ARTICLE INFO

Article history:
Received 1 December 2012
Received in revised form
18 October 2013
Accepted 23 October 2013

Keywords:
Recombinant human Trx-1
Cerebral ischemia
Hippocampus
Neurogenesis
Learning and memory
ERK signaling pathway

ABSTRACT

Cerebral ischemia (CI) can induce loss of hippocampal neurons, causing cognitive dysfunction such as learning and memory deficits. In adult mammals, the hippocampal dentate gyrus contains neural stem cells (NSCs) that continuously generate newborn neurons and integrate into the pre-existing networks throughout life, which may ameliorate cognitive dysfunction following CI. Recent studies have demonstrated that recombinant human thioredoxin-1 (rhTrx-1) could promote proliferation of human adipose tissue-derived mesenchymal stem cells and angiogenesis. To investigate whether rhTrx-1 also regulates hippocampal neurogenesis following CI and its underlying mechanisms, adult mice were subjected to bilateral common carotid arteries occlusion (BCCAO) to induce CI and treated with rhTrx-1 before reperfusion. Mice treated with rhTrx-1 showed shortened escape latencies in Morris water maze by 30 days and improvements in spatial memory demonstrated by probe trial test. Enhanced NSCs proliferation was observed at day 14, indicated by BrdU and Ki67 immunostaining. Doublecortin $(DCX)^+$ cells were also significantly increased following rhTrx-1 treatment. Despite increases in $BrdU^+/NeuN^+$ cells by day 30, the double-labeling to total $BrdU^+$ ratio was not affected by rhTrx-1 treatment. The promotive effects of rhTrx-1 on NSCs proliferation and differentiation were further confirmed in *in vitro* assays. Western blot revealed increased ERK1/2 phosphorylation after rhTrx-1 treatment, and the ERK inhibitor U0126 abrogated the effects of rhTrx-1 on NSCs proliferation. These results provide initial evidence that rhTrx-1 effects neurogenesis through the ERK signaling pathway and are beneficial for improving spatial learning and memory in adult mice following global CI.

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1. Introduction

Cerebral ischemic (CI) and neurodegenerative diseases can induce hippocampal neuron loss of varying severity which are pivotally implicated in the deficiencies of spatial learning and memory (Bendel et al., 2005; Chen et al., 2000; Hartman et al., 2005; West et al., 1994). Although persistent progenitor cell proliferation and neurogenesis commonly follow ischemic periods in the forebrain subventricular zone and hippocampal dentate gyrus (DG), a phenomenon that has been well documented in rodent models, the definite role of neurogenesis following CI remains controversial.

Neurogenesis in response to ischemic neuron loss may be a result of neural stem cells (NSCs) participation in the endogenous repair processes of the adult mammalian brain (Okano et al., 2007). New neurons have been detected in the injured area of previously ischemic tissues, where these new cells were functionally integrated into existing cerebral circuits (Lichtenwalner and Parent, 2006). Conversely, neurogenesis has been suppressed using X-ray irradiation prior to bilateral common carotid arteries occlusion (BCCAO) in gerbil models, resulting in severe spatial learning and memory deficits following ischemia (Raber et al., 2004). These findings underscore the importance of neurogenesis in functional recovery. Enhanced hippocampal neurogenesis also facilitates long-

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http://dx.doi.org/10.1016/j.neuropharm.2013.10.027

term potentiation and improves learning and memory (Abraham et al., 2002; Deng et al., 2010; Schmidt-Hieber et al., 2004). Therefore, facilitation of endogenous neurogenesis may be a promising regenerative strategy for ischemic stroke treatment.

Both direct and indirect approaches have been developed to stimulate proliferation and neurogenesis, attenuate infarct volume, and improve neurological deficits in the ischemic stroke model. Thioredoxin (Trx), a 12-kD oxidoreductase enzyme containing a dithiol-disulfide active site, is a redox-sensitive molecule with pleiotropic cellular effects involved in regulation of proliferation, redox states, and anti-apoptosis (Mukherjee and Martin, 2008). Two isoforms of Trx exist in mammalian cells. Cytosolic Trx-1, which can be translocated into the nucleus under certain circumstances, is the dominant form, and mitochondrial Trx-2 occurs relatively less frequently (Dunn et al., 2010). Endogenous Trx-1 production is induced by ischemia of brain tissues, where it is responsible for alleviation of oxidative damage. Moreover, exogenously administered recombinant human Trx-1 (rhTrx-1) can penetrate the blood brain barrier and exert a neuroprotective effect, as demonstrated in the middle cerebral artery occlusion (MCAO) model (Hattori et al., 2004). In addition to its role as an antioxidant, rhTrx-1 has also been reported to promote proliferation of human adipose tissue-derived mesenchymal stem cells and angiogenesis (Song et al., 2011; Welsh et al., 2002).

While the neuroprotective role of rhTrx-1 on injured neurons is well-established, rhTrx-1 may also promote neurogenesis and facilitate long-term recovery following CI. In order to investigate this hypothesis, the current study applied the BCCAO mouse model to investigate the role of rhTrx-1 in neurogenesis and its potential effect on cognitive deficits in learning and memory following global CI. In addition, the mechanisms associated with rhTrx-1 were tentatively explored.

2. Materials and methods

2.1. Animals and experimental design

Male C57BL/6J mice weighing 25–30 g purchased from the Laboratory Animal Center of the Fourth Military Medical University (Shaanxi, China) were used in the current *in vivo* studies. All experimental protocols complied with the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health (NIH). All experiments were also conducted in accordance with the Fourth Military Medical University Committee on Animal Care.

Mice were provided *ad libitum* access to food and water, and mice were housed in a temperature-controlled room with 12 h light–dark cycles. Experimental procedures for *in vivo* studies are described in Fig. 1. Briefly, mice were randomized into 3 groups: the sham-operated group, the cerebral ischemia plus vehicle (CI + Vehicle) group, and the cerebral ischemia plus rhTrx-1 (CI + rhTrx-1) group. Mice of each group were then equally divided into two subgroups for cognitive function evaluation and neurogenesis detection, respectively.

Mice of the CI + rhTrx-1 group were subjected to transient BCCAO for 20 min and intraperitoneal (i.p.) administration of rhTrx-1 containing 3 mg/kg of the cytosolic form of Trx (Sigma-Aldrich, St. Louise, MO) 10 min before reperfusion, according to the previously published method (Hattori et al., 2004; Tao et al., 2006). The CI + Vehicle group was simultaneously treated with similar BCCAO and i.p. administration of an equal volume of a 0.1 M phosphate buffered saline (PBS) solution as an inert vehicle (pH 7.4).

For neurogenesis analysis, mice were treated with daily 100 mg/kg i.p. injections of 5-bromo-2′-deoxyuridine (BrdU; Sigma-Aldrich, St. Louis, MO) for 4 consecutive days, during the peak of cell proliferation, or from days 9–12 after rhTrx-1 or vehicle treatment, according to the previously reported method (Bernabeu and Sharp, 2000; Liu et al., 1998). Mice of the sham-operated group also underwent i.p. injection of BrdU following sham surgeries. All the mice were sacrificed by days 14 and 30 and tissues were collected for further immunohistochemical study. Behavioral tests were performed 30 days after ischemia. NSCs were harvested from the fetal brain of pregnant Sprague-Dawley rats under deep anesthesia induced by treatment with 300 mg/kg i.p. chloral hydrate between embryonic days 14.5 and 16.5.

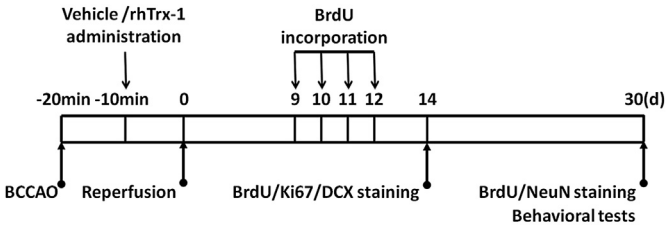

Fig. 1. Graphic presentation of the experimental protocol. The mice brain ischemia model was induced by BCCAO for 20 min. Experimental mice were randomized to receive either vehicle (0.1 M PBS, pH 7.4; i.p.) or an equal volume of rhTrx-1 (3 mg/kg; i.p.) 10 min before reperfusion. BrdU (100 mg/kg; i.p) was administrated daily for 4 consecutive days from 9 to 12 days after ischemia, and mice were then sacrificed for neurogenesis assays at 14 and 30 days post-ischemia. Behavioral testing was conducted 30 days after ischemia. Sham-operated animals were also injected with BrdU after sham surgeries and subjected to behavioral testing prior to sacrifice.

2.2. Transient forebrain cerebral ischemia

Mice were anesthetized with 2% isoflurane using a facemask, and CI was induced by transient BCCAO for 20 min, as described previously (Homi et al., 2003; Tajiri et al., 2004; Wu et al., 2001). Sham-operated animals were treated with the same surgical procedure, with the exception of occlusion. A PeriFlux System 5000 laser Doppler flowmeter (Perimed, Stockholm, Sweden) was used to measure regional cerebral blood flow (rCBF) from the start of anesthetic induction to 5 min after reperfusion (Olsson et al., 2004; Yoshioka et al., 2011). In the current experimental model, only results from mice with a mean CBF successfully reduced to 10% of the pre-ischemic value were used in subsequent data analysis. Rectal temperature was maintained at 37.5 ± 0.5 °C with a heating lamp until mice were revived.

2.3. Arterial blood gas determination

Left femoral arterial catheters were placed in separate groups of sham, CI + Vehicle and CI + rhTrx-1 groups ($n = 5$ in each group) to determine the arterial blood gas (Table. 1). About 0.2 ml of blood

Table 1
Physiologic parameters.

	T (°C)	pH	PaO_2 (mmHg)	$PaCO_2$ (mmHg)
Onset of BCCAO				
Sham	37.0 ± 0.12	7.39 ± 0.02	192.4 ± 0.8	37.5 ± 0.6
CI + vehicle	36.9 ± 0.20	7.40 ± 0.02	193.6 ± 1.1	38.1 ± 0.3
CI + rhTrx-1	37.1 ± 0.18	7.38 ± 0.04	189.5 ± 0.7	36.3 ± 0.4
Onset of reperfusion				
Sham	37.1 ± 0.15	7.41 ± 0.02	196.2 ± 0.5	39.1 ± 0.7
CI + vehicle	37.0 ± 0.11	7.40 ± 0.03	190.8 ± 1.6	38.5 ± 0.6
CI + rhTrx-1	37.1 ± 0.13	9.48 ± 0.04	192.9 ± 1.4	37.6 ± 0.5

Data was shown as mean ± SD. CI + vehicle and CI + rhTrx-1 mice were induced by transient BCCAO for 20 min, while sham-operated mice were treated with the same surgical procedure, with the exception of occlusion. BCCAO = bilateral common carotid arteries occlusion; PaO_2 = arterial oxygen tension; $PaCO_2$ = arterial carbon dioxide tension.

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