Neuroscience Letters 543 (2013) 152–156

Contents lists available at SciVerse ScienceDirect

Neuroscience Letters

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

Injection of neural progenitor cells attenuates decrease in level of connexin 43 in brain capillaries after cerebral ischemia

Yoshiyuki Moriyama, Norio Takagi*, Chisa Itokawa, Kouichi Tanonaka

Department of Molecular and Cellular Pharmacology, Tokyo University of Pharmacy and Life Sciences, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan

HIGHLIGHTS

- Cerebral ischemia induced a sustained decrease in the level of connexin 43 in the isolated brain capillaries.
- The injection of NPCs after cerebral ischemia increased the level of connexin 43 in the isolated brain capillaries.
- Some of the injected NPCs migrated into the blood vessels in the peri-infarct area.

ARTICLE INFO

Article history:
Received 1 February 2013
Received in revised form 5 March 2013
Accepted 21 March 2013

Keywords:
Cerebral ischemia
Gap junction
Brain capillaries
Neural progenitor cells

ABSTRACT

Although functional disruption of the cerebrovasculature, which is called the "neurovascular unit (NVU)", may lead to amplification of ischemia-induced injury, changes in the gap junctional proteins within the NVU and their pathophysiological roles after brain injury remain controversial. We previously demonstrated that the intravenous injection of neural progenitor cells (NPCs) have therapeutic potential for improving the spatial learning dysfunction and depression-like behaviors observed after cerebral ischemia. In this study, we investigated whether severe cerebral ischemia would alter the expression of gap junctional proteins in isolated brain capillaries and examined the effect of intravenous injection of NPCs on the levels of these proteins. Cerebral ischemia induced a sustained decrease in the level of the gap junctional protein connexin 43 (Cx43) in the isolated brain capillaries, whereas the level of aquaporin 4 (AQP-4) was transiently increased. The injection of NPCs increased the level of Cx43 compared that of vehicle in the microsphere embolism (ME) rats, suggesting this decrease to be a possible mechanism for disruption of the astrocyte-endothelial cell interface within the NVU without causing any changes in the level of AQP-4 and N-cadherin. We also demonstrated that some of the intravenously injected NPCs migrated into the blood vessels in the peri-infarct area. These results suggest that the intravenous injection of the NPCs would remodel the NVU after severe cerebral ischemia, which remodeling might be associated with functional improvement following the NPC injection.

1. Introduction

Stroke is a major cause of death and disability and is a highly complex process. Despite a wealth of data about the pathophysiological alterations following a stroke, clinically effective drugs have not been well established. It might be due to a singular focus on ischemia-induced brain injury. Recently, the concept of the "neurovascular unit (NVU)" has arisen as a new model for understanding the pathological alterations elicited by stroke. The NVU is composed of several types of cells, including endothelial cells, pericytes at the capillary level, vascular smooth muscle cells at the arterial level, astrocytes, microglia, and neurons. It has been suggested that the maintenance of the adherens, gap, and tight junctions within the NVU is essential for vascular homeostasis in the central nervous system [16]. In this sense, the gap junctional communication appears to form an important component in the direct cell–cell communication essential for regulating functions in various organs. Although connexin 43 (Cx43), one of the gap junctional proteins, is predominantly expressed in astrocytes in the brain, pairs of Cx43 hemichannels are expressed in pericytes and endothelial cells. Furthermore, Cx43 is also abundant at astrocyte–endothelial cell and astrocyte–neuron interfaces. In addition, N-cadherin is the key adherens junction protein between pericytes and endothelium. This cerebrovasculature has been suggested to play a pivotal role in and serve as the basis of brain function. Thus, functional disruption of the NVU may lead to amplification of ischemia-induced injury. However, the role of the gap junctional communication under ischemic conditions remains controversial [12,17]. In addition, it remains unclear whether the expression of Cx43 in the NVU is altered after cerebral ischemia.

* Corresponding author. Tel.: +81 42 676 4584; fax: +81 42 676 4584.
E-mail address: takagino@toyaku.ac.jp (N. Takagi).

0304-3940/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neulet.2013.03.053

We previously demonstrated that the intravenous injection of neural progenitor cells has therapeutic potential for improving spatial learning dysfunction and depression-like behaviors without any restorative effect on the degeneration of the brain tissue after cerebral ischemia [9–11]. However, it remains to be determined whether the injection of NPCs would affect the expression of Cx43 when given several days after the induction of a cerebral embolism. In this study, we isolated brain capillaries from ischemic rats and investigated whether long-term and severe cerebral ischemia would alter the expression of Cx43 in the isolated brain capillaries. We furthermore examined the effect of intravenous injection of NPCs on the levels of gap junctional protein Cx43 and N-cadherin in brain capillaries after cerebral ischemia, in addition to that of the injection on aquaporin 4 (AQP-4) at the astrocyte–endothelial cell interface.

2. Materials and methods

2.1. Neural progenitor cell (NPC) cultures

NPCs were prepared from gestational Day 14 fetal green fluorescent protein (GFP) transgenic Wistar rats as described previously [11]. The GFP transgenic rats [Wistar-TgN(CAG-GFP)184ys] used in this study were provided by the National Bio Resource Project for the Rat (Kyoto, Japan). The origin and characteristics of the transgenic rats were described earlier [6]. The cells were seeded at a density of 5.0×10^4 cells/cm^2 into non-treated flasks (Nalge Nunc International, New York, NY, USA) containing N-2 plus medium supplemented with 20 ng/ml epidermal growth factor and 20 ng/ml basic fibroblast growth factor. In this study, neurospheres cultured for 6 days *in vitro* were used. The protocol was approved by the Committee of Animal Care and Welfare of Tokyo University of Pharmacy and Life Sciences.

2.2. Model of microsphere-induced cerebral embolism in rats

Male Wistar rats weighing 220–250 g (Charles River Japan Inc., Tsukuba, Japan) were used. The rats were maintained at $23 \pm 1\,^{\circ}C$ in a room with a constant humidity of $55 \pm 5\%$ and a light cycle of 12-h light:12-h darkness. The rats had free access to food and water according to the National Institute of Health Guide for the Care and Use of Laboratory Animals and the Guidance for Experimental Animal Care issued by the Prime Minister's Office of Japan. The study was approved by the Committee of Animal Care and Welfare of Tokyo University of Pharmacy and Life Sciences. Microsphere-induced cerebral embolism was performed by the method described previously [11]. After the rats had been anesthetized with 40 mg/kg sodium pentobarbital, their right external carotid and pterygopalatine arteries were temporarily occluded with strings. Immediately, a needle connected to a polyethylene catheter (TORAY Feeding Tube, Chiba, Japan) was inserted into the right common carotid artery, and then 700 microspheres (45.0 μm in diameter; Polysciences Inc., Warrington, PA, USA), suspended in 20% dextran solution (150 μl), were injected in 20 s into the right internal carotid artery through the cannula. After the injection, the needle was removed; and the puncture wound was then repaired with surgical glue. The non-operated rats were used as naïve control rats in the present study.

2.3. Neurological deficits

Neurological deficits of ME rats were scored on the basis of paucity of movement, truncal curvature, and forced circling during locomotion according to the criteria described previously [5,8]. The score of each neurological deficit was rated from 3 to 0 (3, very severe; 2, severe; 1, moderate; 0, little or none). The rats with a total score of 7–9 points on day 1 after surgery were used in the present study.

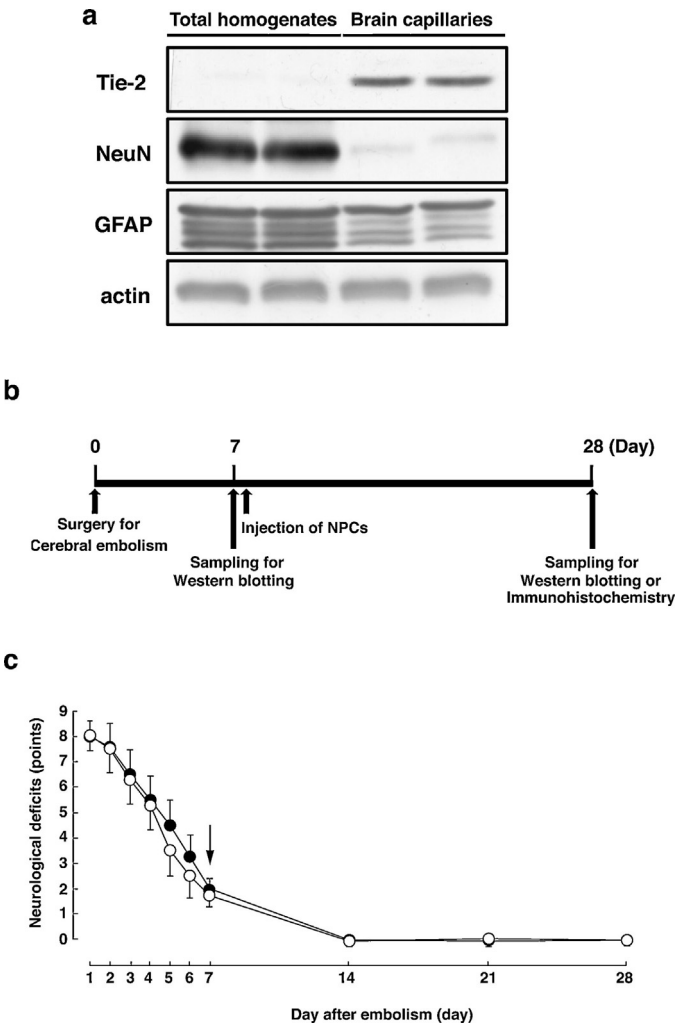

Fig. 1. Characteristics of brain capillaries isolated form a non-operated control rat. (a) Representative immunoblots for Tie2, NeuN, and GFAP, which are markers of endothelial cells, neurons, and astrocytes, respectively, in the total brain homogenates and isolated brain capillaries. (b) Experimental protocol used in this study is depicted. (c) Time courses of change in neurological deficits of vehicle- (closed circles) and NPC-injected (open circles) ME rats. Results are presented as the mean ± S.E.M. ($n = 4$ rats per group). The arrow indicates the time point when vehicle or NPCs was injected.

2.4. Administration of NPCs

Neurospheres were dispersed into single cells in an enzyme solution (123 mM NaCl, 5 mM KCl, 26 mM $NaHCO_3$, 10 mM D-glucose, pH 7.1, containing 3.2 mM $MgCl_2$, 2 mM $CaCl_2$, 1.33 mg/ml trypsin, 0.67 mg/ml hyaluronidase, and 0.2 mg/ml kynurenic acid) and resuspended in DMEM/F12 medium to a final concentration of 1.0×10^6 cells/100 μl. The cell suspension (100 μl) was injected into the right femoral vein on day 7 after the cerebral embolism. Vehicle was injected in a similar manner as the NPCs (for experimental protocol, see Fig. 1b).

2.5. Immunohistochemical assessments

On day 28 after surgery, cerebral ischemic rats were perfused *via* the heart with 4% paraformaldehyde in 0.1 M phosphate buffer. Their brains were quickly removed and immersed in 30% sucrose in 0.1 M phosphate buffer. The brains were then cut into