ELSEVIER

Journal of the Neurological Sciences 240 (2006) 21 – 29

Journal of the Neurological Sciences

www.elsevier.com/locate/jns

Neuroregenerative effects of BMP7 after stroke in rats

Jenny Chou [a], Brandon K. Harvey [a], Chen-Fu Chang [b], Hui Shen [a], Marisela Morales [a], Yun Wang [a,*]

[a] National Institute on Drug Abuse, IRP, 5500 Nathan Shock Drive, Baltimore, MD 21224, USA
[b] Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan

Received 22 November 2004; received in revised form 21 July 2005; accepted 24 August 2005
Available online 19 October 2005

Abstract

Previous reports have indicated that the expression of bone morphogenetic protein-7 (BMP7) is enhanced after ischemic injury in brain. This upregulation may induce endogenous neurorepair in the ischemic brain. The purpose of this study was to examine neuroregenerative effects of BMP7 after ischemia–reperfusion injury. Adult Sprague–Dawley rats were anesthetized with chloral hydrate. Right middle cerebral artery (MCA) was transiently ligated with 10-O suture for 1 h. One day after MCA occlusion, vehicle or BMP7 was infused to the contralateral cerebral ventricle. To identify possible neurogenesis, bromodeoxyurindine (BrdU) was systemically injected on the fourth and fifth days after MCA occlusion. Animals treated with BMP7 showed a rapid correction of body asymmetry and neurological deficits, suggesting BMP7 facilitates recovery after stroke. Animals were sacrificed at 1 month after stroke and brains were analyzed using immunohistological techniques. BMP7 treatment enhanced immunoreactivity of BrdU in the subventricular zone, lesioned cortex, and corpus callosum. These BrdU-positive cells co-labeled with nestin and NeuN. Our behavioral and anatomical data suggest that BMP7 promotes neuroregeneration in stroke animals, possibly through the proliferation of new neuronal precursors after ischemia.
Published by Elsevier B.V.

Keywords: Stroke; Growth factors; Bone morphogenetic protein-7

1. Introduction

Bone morphogenetic protein-7 (BMP7) is a trophic protein in the transforming growth factor-β (TGF-β) superfamily. Both BMP7 and its receptors (BMPRs) are found in neuronal tissue [1]. Several physiological and pathological responses are related to the actions of BMP7 and BMPRs in the central nervous system (CNS). For example, knocking out BMP receptor type 1 (BMPR-1B) resulted in elevated apoptosis in the developing retina [2]. Exogenous application of BMP7 to rat mesencephalic cell cultures increased both the number of tyrosine hydroxylase-expressing cells and dopamine uptake [3]. Pretreatment with BMP7 reduced 6-hydroxydopamine (6-OHDA)-induced toxicity in nigrostriatal dopaminergic neurons [4,5]. After transient cerebral ischemia or brain contusion, BMP7 and BMPRs were upregulated in the cortex [6] and the granule cells of dentate gyrus [7,8]. Moreover, pretreatment with BMP7 prior to general hypoxia or ischemia reduced brain infarction volume and mortality in rats [9,10]. Transplantation of fetal kidney tissue, which contains high levels of BMP-7, reduced cerebral infarction in stroke rats [11]. Taken together, these data suggest that BMP7 is protective against various neurodegenerative processes in the CNS.

Besides its neuroprotective effect, BMP7 promotes functional recovery after stroke. Intracisternal administration of BMP7 1 day after focal cerebral ischemia enhanced recovery of sensorimotor function in the impaired limbs [12,13]. In addition, systemic application of BMP7, given 1 day after middle cerebral artery occlusion (MCAo), decreased body asymmetry and increased locomotor activity from day 7 to day 14 after stroke [6]. These data suggest that BMP7 plays a role in restoring motor function after ischemia. The mechanism of this action is not known.

* Corresponding author. Tel.: +1 410 550 1553; fax: +1 410 550 1425.
E-mail address: ywang@intra.nida.nih.gov (Y. Wang).

0022-510X/$ - see front matter. Published by Elsevier B.V.
doi:10.1016/j.jns.2005.08.015

Activation of BMP signaling is important for cell proliferation and neuronal plasticity. BMP7 promoted DNA synthesis, as visualized by 5-bromodeoxyuridine (BrdU) incorporation in mesencephalic cultured neurons [3]. BMP7 also induced dendritic growth in cultured sympathetic neurons [14]. The expression of netrin, an axon guidance molecule, was suppressed in mice lacking the BMPR-1B (BMPR-1B −/− mice). These knockout mice developed a defect in ganglion cell axon targeting to the optic nerve head [2]. It is thus likely that BMP7 participation in functional neurorecovery is mediated through the formation of new neurons and/or new neuronal connections.

In the present study, we combined functional and anatomical studies to examine the role of BMP7 in stroke rats. We found that intracerebroventricular administration of BMP7, 1 day after MCAo, facilitates the recovery from the neurological deficits in stroke animals. BMP7 increased BrdU immunoreactivity in the corpus callosum and the subventricular zone (SVZ) contralateral to the infarcted side, as well as lesioned cortex. We established the neuronal nature of these BrdU-labeled cells by showing that they contained nestin or NeuN. Our data suggest that BMP7 has neuroregenerative effects in stroke animals. This functional recovery may relate to the genesis of new neural cells in the ischemic brain.

2. Materials and methods

2.1. MCA ligation

Adult male Sprague–Dawley rats ($n=33$) were used for this study. Animals were anesthetized with chloral hydrate (400 mg/kg, i.p. initially and 100 mg/kg every hour). The right MCA was ligated with a 10-O suture using methods previously described [15]. The ligature was removed after 60-min ischemia to generate reperfusional injury. Core body temperature was monitored with a thermistor probe and maintained at 37 °C with a heating pad during anesthesia. After recovery from the anesthesia, body temperature was maintained at 37 °C using a temperature-controlled incubator. Immediately after the recovery from anesthesia, an elevated body swing test [16] was used to evaluate the success of MCAo surgery. All animals used for this study demonstrated prominent motor bias contralateral to the lesioned side.

2.2. Intracerebroventricular injection of BMP7 or vehicle and systemic administration of BrdU

Animals were anesthetized with chloral hydrate 24 h after MCAo. BMP7 (1 μg/1 μl × 25 μl) or vehicle (25 μl, 20 mM acetate/5% mannitol buffer solution) was administered intracerebroventricularly (i.c.v.) through a Hamilton syringe over 10 min. The coordinates for these sites were 0.8 mm posterior to the bregma, 1.5 mm lateral to the midline, −3.7 mm below dura surface (these coordinates correspond to the ventricle contralateral to the ischemic hemisphere). The speed of injection was maintained by a syringe pump at a speed of 2.5 μl/min. The needle was retained in place for 5 min after injection. A piece of bone wax (W810, Ethicon) was applied to the skull defect to prevent the leakage of the solution after injection. BrdU (1%, Sigma, St Louis) was injected on the fourth and fifth day after MCAo at the dose of 0.05 ml/kg (b.i.d, i.p.). The animals were sacrificed and perfused 1 month after stroke.

2.3. Behavioral measurements

2.3.1. Body asymmetry

Body asymmetry was quantitatively analyzed using the elevated body swing test [16]. Rats were examined for lateral movements/turning when their bodies were suspended 20–30 cm above the testing table by lifting their tails. The frequency of initial turning of head or upper body contralateral to the ischemic side was counted in 20 consecutive trials.

2.3.2. Modified Bederson's test

Neurological deficits were evaluated using Bederson's score [17]. In a postural reflex test, rats were examined for the degree of abnormal posture when suspended by 20–30 cm above the testing table. They were scored according to the following criteria.

0. Rats extend straight both forelimbs. No observable deficit.
1. Rats keep the one forelimb to the breast and extend the other forelimb straight.
2. Rats show decreased resistance to lateral push in addition to behavior in score 1 without circling.
3. Rats twist the upper half of their body in addition to behavior in score 2.

2.4. Histochemistry

2.4.1. Triphenyltetrazolium chloride (TTC) staining

One month after reperfusion, animals were anesthetized and perfused intracardially with saline. The brain tissue was then removed and sliced into 2.0-mm-thick sections. The brain slices were incubated in a 2% triphenyltetrazolium chloride (Sigma, St. Louis) for 15 min at room temperature and then transferred into a 4% paraformaldehyde solution for fixation. Because of the liquefication of the infarcted tissue at 4 weeks after MCAo, the ischemic injury on the brain volume was indirectly obtained by the loss of brain tissue. The area of tissue loss in the each slice was measured by subtracting the total area in the right (lesioned) cortex from the total cortical area of the left hemisphere. The volume of tissue loss in each animal was obtained from the product of average slice thickness (2 mm) and sum of the area of tissue loss in all brain slices.