Neurochemistry International 60 (2012) 592–596

Contents lists available at SciVerse ScienceDirect

Neurochemistry International

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

Neuroprotective effects of VEGF administration after focal cerebral ischemia/reperfusion: Dose response and time window

Jiping Yang [a,*], Li Guo [b], Ruichun Liu [b], Huaijun Liu [a]

[a] Department of Medical Imaging, The Second Hospital of Hebei Medical University, Shijiazhuang, Hebei Province, China
[b] Department of Neurology, The Second Hospital of Hebei Medical University, 215 West Heping Road, Shijiazhuang 050000, Hebei Province, China

ARTICLE INFO

Article history:
Received 15 July 2011
Received in revised form 25 January 2012
Accepted 20 February 2012
Available online 28 February 2012

Keywords:
Focal cerebral ischemia
Reperfusion injury
Vascular endothelial growth factor
Neuroprotection
Time window

ABSTRACT

Administration of vascular endothelial growth factor (VEGF) has been shown to increase cerebral blood flow and reduce neurological damage after experimental ischemic brain injury. The purpose of this study was to examine the optimal dose and time window for the neuroprotective effect of VEGF when administrated after focal ischemia/reperfusion injury in rabbits. Focal cerebral ischemia/reperfusion was induced by the middle cerebral artery occlusion (MCAO) method. In a dose response experiment, low (1.25 ng/μL), middle (2.5 ng/μL) and high (5.0 ng/μL) doses of VEGF were administered 2 h after MCAO by the route of perifocal region. The VEGF at a dose of middle (2.5 ng/μL) displayed excellent effects on neuroprotective efficacy for focal cerebral ischemia/reperfusion injury. In another experiment, 2.5 ng/μL VEGF was administered at times varying from 2 to 8 h after MCAO. Infarct volume, water content and neurological deficits were significantly reduced when VEGF was given at 2 and 3 h after injury. The protective effect was less when the same dose was given at the later times. Thus, the present findings indicated that VEGF reduced ischemic neuronal danger with a therapeutic time window within the first 3 h of transient MCAO and may be useful in the treatment of acute ischemic stroke in humans.

1. Introduction

Vascular endothelial growth factor (VEGF) is an angiogenic peptide that also exerts a large number of diverse neuronal effects in the central and peripheral nervous system (Lazarovici et al., 2006). VEGF can remain elevated in the various brain cell types after ischemia (Wang et al., 2004; Hai et al., 2003). Furthermore, upregulation of endogenous VEGF occurs not only in the ischemic areas but also in regions distant from the ischemic site (Stowe et al., 2007). VEGF stimulates neurogenesis, increases survival, promotes growth of neurons and glial cells and protects neuronal tissues from cell death induce by hypoxia or ischemia (Zhu et al., 2005; Chu et al., 2005; Cao et al., 2004; Namiecinska et al., 2005; Greenberg and Jin, 2005; Gora-Kupilas and Josko, 2005). The neuroprotective mechanisms of VEGF-mediated cellular signaling events are based mainly on the activation of the P13K/Akt pathway, inhibition of caspase-3 activation, inhibition of specific potassium currents, as well as enhanced proliferation, migration and differentiation of neuronal progenitors (Gerber et al., 1998; Jin et al., 2001; Qiu et al., 2003; Xu et al., 2003; Tolosa et al., 2008; Sun et al., 2003). We reported recently that VEGF-induced neuroprotective effect by inhibiting extracellular signal-regulated kinase (ERK) signaling pathway and endoplasmic reticulum (ER) stress pathway (Yang et al., 2008, 2009b). These observations showed VEGF has become an interesting potential therapeutic agent preventing neuronal cell death after cerebral ischemia. Obviously, before VEGF could be considered for trials in patients with cerebral brain injury, two issues are important to clarity: first is the optimal dose of the drug and second is the therapeutic time window.

This purpose of this study was to determine the optimal dose and therapeutic time window for administration of VEGF after controlled ischemic brain injury.

2. Experimental procedures

2.1. Animal

A total of 80 male New Zealand White rabbits weighing 2.5–3.0 kg were used. The rabbits were housed in separated cages

Abbreviations: VEGF, vascular endothelial growth factor; MCAO, middle cerebral artery occlusion; ERK, extracellular signal-regulated kinase; ER, endoplasmic reticulum; MCA, middle cerebral artery; MRI, magnetic resonance imaging; DWI, diffusion weighted imaging; ADC, apparent diffusion coefficient; ANOVA, analysis of variance.

* Corresponding author. Address: Department of Medical Imaging, The Second Hospital of Hebei Medical University, 215 West Heping Road, Shijiazhuang 050000, Hebei Province, China. Tel./fax: +86 311 6600 2088.
E-mail address: jipingyang2004@yahoo.com.cn (J. Yang).

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doi:10.1016/j.neuint.2012.02.020

and the room was kept at 24 ± 1 °C temperature and 50–60% humidity, under a 12:12-h light/dark cycle and with access to food and water *ad libitum*. All experimental procedures were approved by the local animal care committee and carried out in accordance with the guidelines of the National Institutes of Health on animal care and the ethical guidelines for investigation of experimental pain in conscious animals. Anesthesia was induced with intravenous injection of 20 mg/kg pentobarbital sodium and if necessary, maintained with a further 5 mg/kg. PE-50 polyethylene tubing was inserted into the left femoral artery for monitoring arterial blood gases, serum glucose, and body temperature before, during and after operation. For pain relief, the surgical wounds were anesthetized in advance by using 2% lidocaine (0.1 ml).

2.2. Surgery

2.2.1. MCA occlusion

The model of intraluminal suture was used for induction of focal cerebral ischemia, as formerly described by Yang et al. (2008, 2009a). Briefly, the left common carotid, internal carotid, and external carotid arteries were exposed via a midline incision in neck. Then left common and external carotid arteries were ligated proximally (near the bifurcation) with 4–0 surgical sutures. A guide wire (RF SP26137M, TERUMO, Japan) with a diameter of 0.53 mm was inserted into internal carotid artery from distal common carotid artery until the tip occluded the origin of the middle cerebral artery (MCA). The placement of guide wire in the MCA was confirmed by a contact X-ray. The guide wire was maintained in place for 2 h and then withdrawn to allow reperfusion. Magnetic resonance imaging (MRI) measurements were performed in all rabbits between 1.75 and 2 h after MCA occlusion (MCAO).

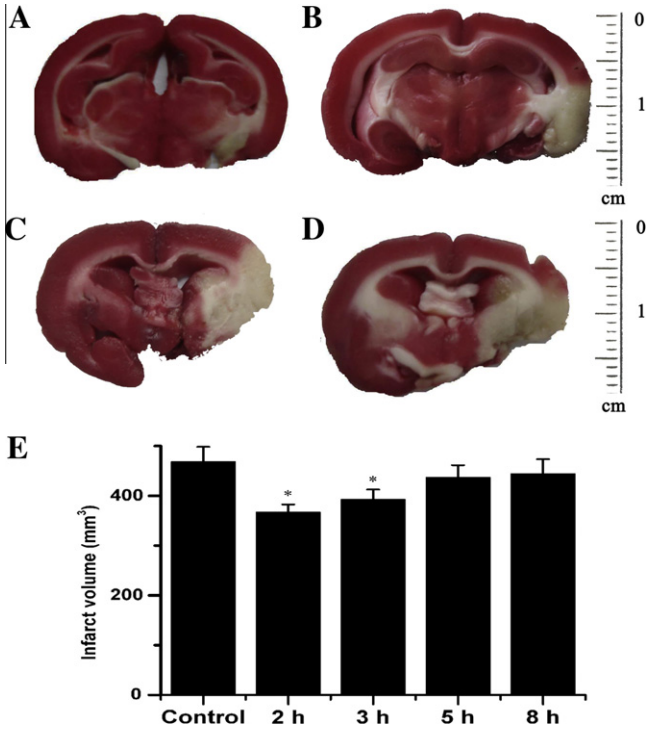

Fig. 1. Postmortem TTC staining and quantitative effects of VEGF on infarct volume after MCAO. At 72 h after MCAO, a significant reduction of infarct volume was found when was administered at 2 (A) or 3 h (B) after MCAO, compared with saline-treated group (D, $P < 0.01$). There was no significantly difference between treatment of VEGF 5 or 8 h (C) post MCAO and saline-treated control (D, $P > 0.05$). (E) Quantitative effects of VEGF on infarct volume after MCAO (Mean ± SD). *vs. saline-treated control group $P < 0.01$.

2.3. Treatment groups

2.3.1. Dose response experiment

To study the effect of different doses of VEGF, a total of 40 male New Zealand White rabbits were randomly assigned to one of the following doses: none (saline-treated group), low (1.25 ng/μL), middle (2.5 ng/μL) and high (5.0 ng/μL). Rabbits received intracerebral microinjection of 50 μL VEGF 165 or same volume of saline 2 h after MCAO by the route of perifocal region. After 2 h of MCAO and 70 h of reperfusion, neurological deficits, infarct volume and water content were measured.

2.3.2. Time window experiment

To study the time window for the neuroprotective effect of VEGF administration, a total of 40 rabbits were assigned to following postischemia times for the administration of a single dose: 2, 3, 5 and 8 h. Rabbits received intracerebral microinjection of 50 μL VEGF 165 at different time points 2, 3, 5 or 8 h post MCAO ($n = 10$ per time point) by the route of perifocal region. After 2 h of MCAO and 70 h of reperfusion, neurological deficits, infarct volume and water content were measured.

2.4. MRI protocol

Diffusion weighted imaging (DWI) was conducted to document injury and to provide a guide for intercerebral injection and dissect tissues from ischemic regions (Fig.2). All animals were imaged in a 1.5-T scanner (Toshiba Visart, Japan), with quadrature knee coil. DWI was performed using a spin-echo echo-planar imaging sequence (TR = 12,000 ms, TE = 108 ms, NA = 3, 2 different *b*-values [$b = 0$ and $b = 900$ s/mm^2], FOV = 16.5 × 16.5 cm, matrix = 96 × 96, 5 slices, slice thickness = 5 mm). If hyperintensity was observed in the MCA territory, apparent diffusion coefficient (ADC) maps were constructed by acquiring a set of five images with increasing diffusion gradient amplitude. The ADC-based measurements of the size of the ischemic core and penumbra were performed as follows: the ranges of the lowest ADC values and ADC values in the matching contralateral anatomic regions were derived from the ADC maps such that the region with ADC values lower than the mean of the lowest ADC values plus 1 SD was referred to as the core (Manabat et al., 2003). The region with ADC values higher than the mean of the ADC values plus 1 SD in the core but lower than the mean of normal ADC values minus 2 SDs in that region was referred to as the ischemic penumbra (Manabat et al., 2003).

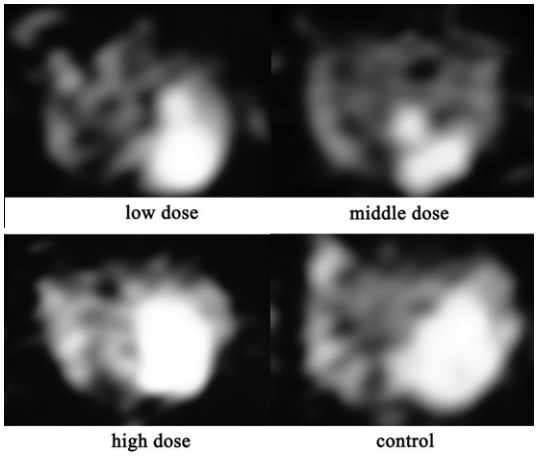

Fig. 2. Representative DWI images assessed at 72 h after MCAO.