Experimental Research

Effects of intraventricular infusion of vascular endothelial growth factor on cerebral blood flow, edema, and infarct volume*

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Summary

Background. Therapeutic cerebral angiogenesis, utilizing angiogenic factors to enhance collateral vessel formation within the central nervous system, is a potential method for cerebral revascularization. A prior dose-response study determined that intracerebroventricular infusion of vascular endothelial growth factor (VEGF) increases vascular density with minimal associated brain edema at a concentration of 5 μg/ml. The purpose of this study was to assess effects of intracerebroventricular infusion of VEGF (5 μg/ml) on cerebral blood flow, infarct volume, and brain edema after ischemia.

Methods. Recombinant human VEGF165 was infused into the right lateral ventricle of rats with an osmotic minipump at a rate of 1 μl/hr for 7 days. Control animals received vehicle only. Ischemia was produced by transient (2 hours) middle cerebral artery occlusion (MCAO). After MCAO, cerebral blood flow was determined with the indicator fractionation technique: infarct volume was assessed with 2,3,5-triphenlytetrazolium chloride staining, and brain edema was determined by measuring brain water content.

Findings. Cerebral blood flow was not significantly different in animals treated with VEGF compared to controls. There was a significant reduction in total infarct volume after temporary MCAO in VEGF-treated animals compared to controls (163 ± 37 mm³ vs. 309 ± 54 mm³, P < 0.05). Brain water content after transient MCAO was also significantly reduced in VEGF-treated animals compared to controls (80.9 ± 0.7% vs. 83.3 ± 0.6%, P < 0.05).

Interpretation. Intracerebroventricular infusion of VEGF165 (5 μg/ml) decreases infarct volume and brain edema after temporary MCAO without a significant increase in cerebral blood flow. These results indicate that VEGF may have a direct neuroprotective effect in cerebral ischemia.

Keywords: Angiogenesis; vascular endothelial growth factor; brain edema; cerebral ischemia.

Introduction

Therapeutic angiogenesis, utilizing angiogenic factors to augment collateral circulation, is an active area of investigation for patients with peripheral arterial occlusive disease [2] and myocardial ischemia [8, 11, 12]. For patients with cerebral hemodynamic compromise, provision of angiogenic factors to the brain may be a viable method to augment current methods of surgical and endovascular cerebral revascularization. Most research in therapeutic angiogenesis has centered on vascular endothelial growth factor (VEGF) [3, 21]. VEGF is a powerful endothelial mitogen that also increases vascular permeability. Plans for the use of VEGF in the central nervous system must consider both effects on angiogenesis as well as the potential for brain edema formation.

Intracerebral injection of VEGF leads to the formation of a cluster of abnormal-appearing blood vessels [13]. A dose-response study in this laboratory utilizing intraventricular infusion of VEGF165 at a rate of 1 μl/hr for 7 days at several different concentrations found that a concentration of 5 μg/ml was the minimum concentration required to produce a significant increase in vessel density in the rat [4]. At 5 μg/ml, there was an increase of 17 to 21% in vessel density in the cerebral cortex compared to control animals infused with vehicle only. At this dose there was also an increase in capillary permeability, which was limited to the diencephalon ipsilateral to the infusion, but brain water content was not significantly in-
increased. A higher dose of 25 μg/ml produced a greater increase in vascular density, but also led to significant ventriculomegaly, possibly due to a VEGF-induced increase in cerebrospinal fluid production.

This study was designed to examine whether the limited degree of angiogenesis induced by intraventricular infusion of 5 μg/ml of VEGF165 would be sufficient to increase cerebral blood flow during middle cerebral artery occlusion in the rat and reduce infarct volume. In addition, it also examined whether any such beneficial effect might be offset by increased cerebral edema.

Methods and material

Procedures using laboratory animals were approved by the Institutional Animal Care and Use Committee.

VEGF infusion

Osmotic minipumps designed to deliver 1 μl/hr for 7 days (Model 2001, Alza) were filled with a solution containing human recombinant VEGF165 (Sigma) in 0.1 M PBS with 0.1% BSA, and each was attached to a brain infusion cannula. VEGF165 was infused at a concentration of 5 μg/ml. Control pumps contained the vehicle only. The filled pumps were primed by incubation overnight in sterile saline. Adult Sprague-Dawley rats (219–230 g) were anesthetized with intraperitoneal ketamine (50 mg/kg) and xylazine (10 mg/kg). After a skin incision over the center of the skull, a pocket was formed over the neck and scapulae to hold the minipump. A 2 mm hole was drilled in the skull 0.6 mm posterior to the coronal suture and 1.2 mm to the right of the sagittal suture. The cannula was placed in the brain to a depth of 4.5 mm from the outer surface of the skull, and a sterile screw was inserted. The cannula was cemented in place and the incision was sutured. After 1 week, the animals were anesthetized for middle cerebral artery occlusion (MCAO).

Middle cerebral artery occlusion

Temporary (2 hours) occlusion of the middle cerebral artery (MCA) was achieved using the suture method of Longa and colleagues [7]. Briefly, anesthesia was induced with intraperitoneal ketamine (50 mg/kg). Body temperature, but not brain temperature, was maintained at 37 °C. In addition, it also examined whether any such beneficial effect might be offset by increased cerebral edema.

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