The Effect of Sympathetic Denervation on Cerebral Arteriogenesis After Chronic Cerebral Hypoperfusion

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ABSTRACT

Objective: To explore the effect of perivascular sympathetic nerve on cerebral collateral arteriogenesis in chronic cerebral hypoperfusion models of rats.

Materials and Methods: Chronic cerebral hypoperfusion model was established by right common carotid artery ligation for 8 weeks, while sympathetic denervation was performed by superior cervical ganglionectomy. The male Sprague-Dawley rats were randomly divided into 4 groups including sham group ($n = 21$), denervation group ($n = 21$), artery ligation group ($n = 21$) and combined group with both artery ligation and denervation ($n = 21$). After 8 weeks of surgery, the rats in each group were randomly divided into 3 subgroups including subgroup A ($n = 7$), subgroup B ($n = 7$) and subgroup C ($n = 7$). The 3 subgroups were subjected to latex perfusion, permanent right middle cerebral artery occlusion and immunohistochemical staining, respectively.

Results: The diameters of right leptomeningeal anastomoses in artery ligation group significantly enlarged compared with sham group. When sympathetic denervation was performed in the presence of artery ligation, diameter of collateral vessel decreased, although larger than in sham group. After 8 weeks of permanent right middle cerebral artery occlusion, the cerebral perfusion over the right middle cerebral artery area in combined group was significantly lower than in artery ligation group, although both were higher than in denervation group and sham group. Triphenyltetrazolium chloride staining showed that cerebral infarct volume in combined group was significantly larger than in artery ligation group, and smaller than in denervation group and sham group. Neurologic functional scoring showed that scores in combined group were significantly higher than in artery ligation group, and lower than in denervation group and sham group. Immunohistochemical staining for α-smooth muscle actin showed that compared with sham group, tunica media thickness of right leptomeningeal anastomoses in artery ligation group increased significantly. Thickness in combined group was thinner than in artery ligation group, although thicker than in sham group.

Conclusions: Perivascular sympathetic denervation can impair the cerebral collateral arteriogenesis under condition of chronic cerebral hypoperfusion.

Key Indexing Terms: Chronic cerebral hypoperfusion; Cerebral collateral arteriogenesis; Perivascular sympathetic nerve.
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INTRODUCTION

Reduction of cerebrovascular reserve because of major artery occlusion leads to cerebral hypoperfusion, which tremendously increases the risk of ischemic stroke. At this time, arteriogenesis is stimulated aimed at restoring cerebrovascular reserve, and determines the severity of subsequent ischemic injury of the brain.[1-3] Arteriogenesis refer to the maturation and enlargement of the pre-existing small vessels through vascular remodeling; especially the integration of smooth muscle cells (SMCs). This style of neovascularization represents a promising approach for ischemic stroke. However, abnormal and excessive vascularization seen in cancer and blinding ocular vasoproliferative disease, could promote disease progression.[4,5] So, controlled vascularization is necessary to restore tissue structures and functions.

The angiogenic process requires intact endothelial and peripheral nervous systems. Nerves and blood vessels establish intricate branching network in every organ that share anatomical and functional characteristics. The alignment of nerves and blood vessels depends on its reciprocal interaction in development. Genetic studies demonstrated that blood vessel patterning was directed by nerves in developmental embryonic limb skin, and arterial differentiation is dependent on the presence of nerves.[6,7] In addition, various neurotransmitters have been demonstrated to be involved in process of neovascularization including migration and proliferation of endothelial cells and vascular SMCs.[8] So, we proposed that perivascular nerves may play critical roles in arteriogenesis under condition of cerebral hypoperfusion.

In the present study, cerebral hypoperfusion was constructed by right common carotid artery (rCCA)

occlusion. Aimed at exploring effect of perivascular sympathetic nerve on arteriogenesis, morphologic changes of collateral leptomeningeal anastomoses was observed after sympathetic denervation. In addition, a series of functional indexes were also tested in hypoperfused animals subsequent to permanent right middle cerebral artery occlusion (pMCAO) including cerebral perfusion, infarct volume and neurologic functional scoring.

MATERIALS AND METHODS

Animals and Grouping

Male adult Sprague-Dawley rats weighing between 250 g and 280 g (Experimental Animal Center of Third Military Medical University, China) were randomly divided into 4 groups including artery ligation group ($n = 21$), denervation group ($n = 21$), combined group ($n = 21$) and sham group ($n = 21$). After 8 weeks of surgery, the rats in each group were randomly divided into 3 subgroups including subgroup A ($n = 7$), subgroup B ($n = 7$) and subgroup C ($n = 7$). The diameter of leptomeningeal anastomoses was observed in subgroup A after latex perfusion; rats in subgroup B were subjected to pMCAO. Cerebral perfusion over the right middle cerebral artery (MCA) area was measured. Cerebral infarct volume was tested by triphenyltetrazolium chloride (TTC) staining at 24 hours postoperation. Immunohistochemical staining was carried out in subgroup C aimed to observe the thickness of the right leptomeningeal anastomoses. All the experimental protocols and animal handling procedures were approved by the Institutional Animal Care and Use Committee of Third Military Medical University, China.

Animal Model

The rats were anesthetized with an intraperitoneal injection of 4% chloral hydrate (350 mg/kg). Chronic cerebral hypoperfusion model was established by rCCA for 8 weeks,[9,10] while denervation was performed by superior cervical ganglionectomy (SCG×),[11] and the ipsilateral blepharoptosis(drooping of the upper eyelid) is used as indicator of the successful removal of the SCG. Artery ligation group only received rCCA ligation. Denervation group only received SCG×. Combined group received both rCCA ligation and SCG×. Sham-operated rats were as control.

Visualization of Vessels by Latex Perfusion

After 8 weeks of rCCA or SCG× or after both, latex perfusion was performed to visualize leptomeningeal anastomoses in each subgroup A. Under deep chloral hydrate anesthesia, the right atrium of the heart was incised to allow for venous outflow. The aorta was cannulated and injected at 150 mm Hg first with 5 mL of saline and then with 5 mL of latex compound that was a mixture of white latex (Chongqing Latex Product Inc, China) and a small amount of carbon black (50 μL/mL, Boss Inc, China). Then the brain was removed carefully from the skull, and photographs were taken of the dorsal and ventral brain surfaces by stereo microscopes (SZX7, Olympus, Japan). The diameter of the leptomeningeal anastomosis was measured at the point of confluence between the distal MCA and the distal anterior cerebral artery (ACA) or between the distal MCA and the distal posterior cerebral artery. At least 8 measurements of the diameters were taken on each hemisphere, and the average of all these measurements was considered as the average diameter of the anastomosis.[12]

pMCAO

The right MCA was occluded by an intraluminal filament method after measurement of cerebral perfusion in each subgroup B.[13] With the animal in a secure supine position, a midline incision was made and the rCCA, external carotid artery and internal carotid artery were exposed. The nylon filament (2636-A4, Beijing Cinontech Co Ltd, China) was introduced through the rCCA incision and advanced through the internal carotid artery at a length of approximately 18 mm from the bifurcation of the rCCA. The nylon filament was then tied tightly. Rats were returned to their cages after surgery.

Measurement of Regional Blood Flow by Laser-Doppler Flowmetry

In subgroup B, the skull was exposed after general anesthesia. A Laser-Doppler Flowmetry (VMS-LDF1, Moor, UK) guide was fixed to the skull 3.5 mm lateral to bregma with dental cement for assessment of the right MCA area.[14] The value measured before rCCA or SCG× or before both was considered as the baseline value. Changes in regional cerebral blood flow (rCBF) were expressed as the percentage change from baseline.

Neurologic Function Scoring

Neurologic function scoring were graded on a scale of 0-5 24 hours after pMCAO in subgroup B. The criteria were as follows: grade 0 = no observable neurologic deficits, grade 1 = failed to extend right forepaw, grade 2 = circled to the right, grade 3 = fell to the right, grade 4 = could not walk spontaneously and grade 5 =dead. The investigator who scored was unaware of the treatments that the animals received.

TTC Staining

Infarct volume was measured by staining with 2% 2,3,5-TTC (Sigma, St Louis, MO) at 24 hours after pMCAO.[15] Briefly, the rats were anesthetized and transcardially perfused with sterile saline and paraformaldehyde as previously described. Serial coronal sections (2-mm thick) were sliced from the adult rat brain matrix (RWD Life Science Co Ltd, San Diego, CA). The slices