The effect of chronic cerebral hypoperfusion on stroke severity and arteriogenesis

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ABSTRACT

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It has been reported that patients with atherothrombotic stroke have smaller infarctions and less severe neurological deficits than those with cardioembolic stroke. Patients with atherothrombotic stroke are more likely to have significant stenosis of the artery proximal to an occlusion, which might have caused chronic cerebral hypoperfusion prior to the stroke. Chronic cerebral hypoperfusion may induce the adaptive growth of preexisting collateral arteries (arteriogenesis), and this may contribute to less severe stroke. To prove this hypothesis, in stroke patients with middle cerebral artery (MCA) occlusion, infarct sizes and severity of initial neurological deficits were compared between those with severe stenosis of the tandem internal carotid artery (ICA) proximal to the site of occlusion and those without the above mentioned stenosis. Infarct

sizes were smaller (P=0.03) and neurological deficits were milder (P<0.01) in those with ICA stenosis. In the second part of experiments, chronic cerebral hypoperfusion was induced in the Sprague-Dawley rat by bilateral common carotid artery ligation (BCAL). MCA occlusion/reperfusion (MCAO/R) using a nylon suture model was introduced 4 weeks after the BCAL (BCAL-MCAO) or the sham operation (Sham-MCAO). Infarct sizes measured after 2,3,5-triphenyltetrazolium chloride staining were significantly smaller in the BCAL-MCAO group (P=0.04). The presence of arteriogenesis was examined by postmortem angiography using the latex-perfusion method. The rats with BCAL had larger diameters of the bilateral posterior cerebral arteries, the bilateral posterior communicating arteries, and the basilar artery (BA) and showed the more tortuous BA than those with sham operation (P<0.01). The vessel density of the middle and posterior portions assessed in the coronal sections of the brains was higher in the rats with BCAL (P<0.05). In conclusion, stroke was less severe when the vicinity subjected to infarction was preconditioned by chronic cerebral hypoperfusion, and arteriogenesis might contribute to this response.

Key words: chronic cerebral hypoperfusion, middle cerebral artery occlusion, acute cerebral infarction, arteriogenesis

The effect of chronic cerebral hypoperfusion on stroke severity and arteriogenesis

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I. INTRODUCTION

Patients with severe stenosis of the extracranial or intracranial artery have often been considered to bear a higher risk of stroke development and a risk of severe stroke if it develops. The risk of stroke development usually increases according as the degree of arterial stenosis increases, as it has been demonstrated in major trials for carotid endarterectomy, and a surgery is beneficial for reducing stroke in patients with severe carotid stenosis.[1, 2] However, as for those with extremely severe carotid stenosis, the risk of stroke decreases paradoxically and the surgery is of no benefit to them.[3-6] These

patients are characterized by the decrease of blood flow distal to the stenosis with the presence of collateral pathways. In addition to the low stroke risk, these patients may have less severe stoke. In the new England medical center posterior circulation registry, the patients with the most severe occlusive disease had the best outcomes.[7] These somewhat paradoxical observations of patients with severe atherosclerosis of the carotid and vertebrobasilar arteries suggest that, when the arterial stenosis is so severe that the brain area supplied by the stenotic artery is hypoperfused chronically, the risk and severity of stroke occurring in the vicinity decrease. Although the durability of collateral circulation has been suggested as being responsible for these observations, the exact mechanism remains yet to be solved.

Similarly, patients with atherothrombotic stroke, when compared with those with cardioembolic stroke, have been reported to have better neurological outcome and smaller infarction volume.[8, 9] The atherothrombotic stroke patients are more likely to have previous transient ischemic attacks or minor strokes, and longstanding atherosclerotic stenosis of extracranial or intracranial arteries, which may cause chronic sublethal hypoperfusion to the brain areas, and adaptive cellular and vascular responses. Again, growth of collateral pathways by chronic hypoperfusion was assumed to contribute to favorable outcomes in atherothromboltic stroke patients. However, to our best knowledge, there has been no directly comparative study of stroke severity or the presence of

collateral circulations between patients with atherothrombotic and cardioembolic infarctions who were subjected to occlusion at the same arterial segment.

Occlusion of the artery at the same segment does not produce the same size of an infarction in individual patients. For example, when the proximal portion of the middle cerebral artery (MCA) is occluded, some patients demonstrate large infarctions encompassing the entire territory supplied by the MCA, which often leads to death, while some have a small infarction limited to the striatocapsular or cortical area. Survival of the brain tissues and infarct sizes depend on many factors including systemic, hemodynamic, and hemorrheologic conditions. Local factors in the brain include the degree of decrease in cerebral blood flow, the size of thrombus or embolus, the presence of collateral circulations, the lag time from the cessation to restoration of blood flow by spontaneous or pharmacological thrombolysis when occlusion occurs, and the acquisition of ischemic tolerance before the infarction.

The adaptive vascular responses following arterial occlusive diseases involve arteriogenesis and angiogenesis. Arteriogenesis is defined as growth of collaterals from pre-existing arterioles for the compensation of occluded arteries. Angiogenesis differs from arteriogenesis as it means formation of new capillaries through sprouting pre-existing capillaries triggered by ischemia.[10-13] Arteriogenesis provides more potent rescue flow upon arterial occlusion

because it has been known that it increases blood flow maximally 10- to 20-folds while angiogenesis does maximally 1.5- to 1.7-folds in the limb ischemia models.[13] In the cerebral vasculature, few studies have examined the presence of arteriogenesis following hypoperfused condtions.[14-16]

This study aimed at proving the hypotheses that chronic cerebral hypoperfusion is protective against subsequent severe ischemic insults both in human stroke and relevant animal models, and that arteriogenesis induced by chronic cerebral hypoperfusion may contribute to this protective process.

II. MATERIALS AND METHODS

1. Stroke severity in patients with MCA occlusion with or without tandem carotid stenosis

A. Patients

Patients with acute MCA infarction and occlusion of horizontal segment (M1) of the relevant MCA, who were admitted to Neurology Department from 2000.7 to 2003.6 and were given complete evaluation including diffusion-weighted MRI (DWI) and angiography, were selected from the Yonsei Stroke Registry.[17]

After excluding those treated with thrombolytics, patients were grouped into 2 groups based on Trial of ORG 10172 in the Acute Stroke Treatment (TOAST) classification: a group with more than 50% stenosis of the relevant proximal tandem artery (LAA) and a group with cardiac embolism (CE).[18] The National Institute of Health Stroke Scale (NIHSS) score on admission, the demographic characteristics such as age, sex, smoking, and medical history of hypertension, diabetes mellitus (DM), cardiac disease, and previous stroke, were compared between the two groups.

B. Measurements of infarction volume

MRI was performed by a 1.5-T system (Signa Horizon, GE Medical Systems, Milwaukee, WI, or Gyroscan Intera, Philips Medical Systems, Best, the Netherlands) with multi-slice echo-planar imaging technique to acquire DWI. The imaging parameters of DWI were as follows: 3400/60/4 (repetition time, msec/effective echo time, msec/excitation), 24 cm field of view, 5/2 mm slice thickness/gap, and 128×128 matrix. B values were 0 and 1000 s/mm^2. For the acquisition of apparent diffusion coefficient, the images were applied to the x, y, and z directions. Acute cerebral infarction was defined as area of high signal intensity on the DWI.

Areas of acute infarctions demonstrated on DWI were measured by a

neuroradiologist who was not aware of the study purpose. Infarction volumes were calculated by multiplying manually contoured hyperintense region by the slice thickness plus the intersection gap with the aid of the Scion image software.

2. The effect of chronic cerebral hypoperfusion in the rat on stroke severity and arteriogenesis

A. Experimental animals and preparation

Male Sprague-Dawley (SD) rats weighting 250 to 340 g were used.[19-21] The care and use of laboratory animals in this experiment were based on the Guidelines and Regulations for Use and Care of Animals in Yonsei University. All animals were allowed free access to food and water. The environmental temperature was maintained at 22.0±2.0 ℃, the humidity at 50±10%, the noise level below 40-50 phons, and the light cycle at 12 hours on/12 hours off. A barrier system that had regular pad change (twice a week), and monitoring of microorganism for specific pathogen free animal were used for the operative procedure. For the operative procedures, the animals were anesthetizes with 5% isoflurane delivered with mixed gas of 30% oxygen and 70% nitrous oxide. Anesthesia was maintained with 2% isoflurane. During the operative procedures,

body temperature was monitored continuously with a rectal probe and was maintained at 37.0±0.5 ℃ by a heating pad (Harvard Apparatus, Holliston, MA, USA). And hemoglobin, pH, PO_2, PCO_2, and mean arterial pressure (MAP) were measured before and after induction of BCAL and MCA occlusion with the use of a femoral artery catheter (OPTI critical care analyze, AVL, AVL Scientific Corporation, Roswell, Georgia, USA). Regional cerebral perfusion (rCP) was determined in the territory of the MCA by laser Doppler flowmetry (LDF) (BLF 21 laser Doppler flowmeter, Transonic System Inc., Ithaca, New York, USA). For the placement of a LDF probe with 1.2 mm diameter tip, the skull around bregma was exposed and drilled 2 to 3 mm in diameter at the point 1 mm posterior to bregma and 3 mm left of the midline with a dental burr. The measurements before BCAL were regarded as the 100% controls.

B. Induction of chronic cerebral hypoperfusion

Chronic cerebral hypoperfusion was induced by means of BCAL as previously described.[19-21] Briefly, after making a midline cervical incision, the common carotid arteries (CCAs) were exposed bilaterally and were double-ligated with 5-0 black silk sutures. The sham SDs underwent the same operation, except that BCAL was not performed. The groups of animals — those that underwent BCAL (BCAL-Control and BCAL-MCAO) and those that

underwent the sham operation (Sham-Control and Sham-MCAO) — were then bred for 4 weeks before the second operation.

C. Induction of acute focal cerebral ischemia and reperfusion

The animals that underwent BCAL (BCAL-MCAO) and those that underwent the sham operation (Sham-MCAO) were subjected to MCAO/R using a nylon suture model.[22, 23] In short, under general anesthesia with 2% isoflurane, the left CCA was exposed through a midline neck incision and was carefully isolated. After dissection, ligation, and coagulation of the external carotid artery and its braches, the internal carotid artery (ICA) was isolated. The ICA was carefully separated from the adjacent vagus nerve, and the pterygopalatine artery was ligated. The CCA was double-ligated with 5-0 black silk in the previously sham-operated animals. Next, in both groups of animals, 5-0 black silk was tied loosely around the ICA, and a microvascular clip was placed across the ICA. A 4-0 nylon monofilament with its tip rounded near a flame and coated with 0.1% poly-L-lysine (Sigma, St. Louise, MO, USA), was introduced into the ICA through a cut. After tightening the silk, which was loosely tied around the ICA, and displacing the clip, the monofilament was advanced into the ICA approximately 23 mm distal to the carotid bifurcation. The incision was closed leaving 1 cm of the nylon suture protruding. Reperfusion was achieved by

pulling back the suture until resistance was felt. The animals were subjected to MCAO for two hours and reperfusion for 22 hours. The sham animals that underwent BCAL (BCAL-Control) and those that underwent the sham operation (Sham-Control) were subjected to the same operation, except the advance of the monofilament and reperfusion.

D. Evaluation of ischemic damage

(A) Motor disability test

Neurologic evaluation was performed using a 5-point scale, which was modified from the scale used by Longa et al.[23] Four items were evaluated and 1 point was given to each item when an animal had a deficit, then summed for a total score. They were (1) failure to grasp the edge of a table when an animal was hung by its tail, (2) failure to extend the right forepaw fully, (3) a circling motion toward the paretic side when attempting to walk, and (4) falling to the lateral side when pushed gently. Thus, an animal without a neurological deficit scored 0 while that with maximal deficits scored 4 points.

(B) Measurements of infarction volume

Twenty-four hours after MCAO/R, the animals were sacrificed by transcardiac perfusion using a peristaltic pump under deep anesthesia with intraperitoneal urethane injection. The brains were cut coronally into 2-mm-thick blocks using a rat brain matrix, incubated for 15 minutes in 2% 2,3,5-triphenyltetrazolium chloride solution at 37 ℃, fixed with 4% formalin solution, and scanned. The stainless areas and the whole brain areas of each section were measured using the Scion image software and summed up. For achieving infarction volume, the ratio of the total infarction areas to the whole brain areas was calculated and expressed as percent.

E. Evaluation of arteriogenesis

After 2 hours of MCAO and 22 hours of subsequent reperfusion, the animals were anesthetized with 2% isoflurane. Lethal dose of papaverine hydrochloride (40-50 mg/kg body weight) was injected through left external jugular vein. Postmortem angiography was performed by a modification of the Coyle's method.[24, 25] Briefly, white latex solution, mixed with carbon black ink and warmed to 37 ℃, was injected through the ascending aorta to visualize cerebral vessels. During this procedure, the descending thoracic aorta was clipped. The rat was decapacitated 30 minutes after latex injection. The brain was fixed with 4% formalin solution, and photographed using a stereozoom microscope with a

camera.

The presence of arteriogenesis was examined both on the surface of the brain and in the parenchyma. On the view of the ventral surface of the brain, diameters were measured at the most proximal portion of MCA, the middle portion of the posterior communicating artery (PcomA), the precommunicating segment and the postcommunicating segment of the posterior cerebral artery (PCA), and the middle portion of the basilar artery (BA) by using the Scion image software (Figure 1). Vessel tortuosity (VT), which was based on the method of a previous report, was defined as the ratio of the vessel length to the straight distance between the two vessel ends (Figure 1).[24] The VT of the BA was calculated using the Scion image software.

After measurement of the vessel diameter and length, the brains were embedded in Tissue-Tek OCT compound (Miles Inc., Elkahart, IN, USA) and frozen in 2-methylbutane/dry ice. For measurements of blood vessels in the parenchyma, 40-um-thick coronal sections using cryostat were obtained at approximately 1, 5, 9, and 13 mm from the frontal pole of the brain, and mounted on the slide. Vessel diameters and numbers were measured using the Computer assisted stereological toolbox system (Olympus Danmark A/S, Albertslund, Denmark). Vessel Density (VD) was expressed as the vessel numbers per the unit area (mm^2).

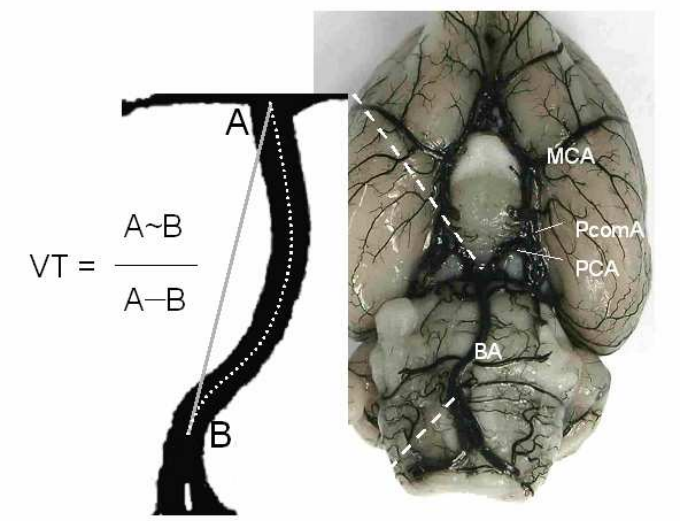

Figure 1. Cerebral arteries on the ventral surface of a rat brain and the definition of vessel tortuosity. Vessel tortuosity (VT) was defined as the ratio of the vessel length (a dotted curved line; A~B) to the straight distance (a solid line; A—B) between the two end points of the vessel (A and B). MCA, middle cerebral artery; PcomA, posterior communicating artery; PCA, posterior cerebral artery; BA, basilar artery.

3. Statistical analysis

The Mann-Whitney *U* test was used for a comparison of age, initial NIHSS, and the infarction volumes between the two patient groups and a comparison of the infarction volumes and neurological deficits between sham-MCAO and BCAL-MCAO. The Kruskal-Wallis test was used for a comparison of

hemoglobin, pH, PO_2, PCO_2, MAP, rCP between sham-MCAO and BCAL-MCAO and vessel diameters, VT, and VD among Sham-Control, Sham-MCAO, BCAL-Control, and BCAL-MCAO. The Fisher's exact test was used for a comparison of sex, hypertension, DM, smoking, cardiac embolic sources, and previous stroke between the two patient groups. All the measurements were expressed as mean ± standard deviation. Statistical significance was set at $P<0.05$. Statistical analyses were performed with SPSS (version 10.0) software.

III. RESULTS

1. Stroke severity in patients with MCA occlusion with or without tandem carotid stenosis

A. Demographic characteristics

During 3-year period from July 2000, 996 patients with acute ischemic stroke were admitted to the Neurology Department. Among them, 41 patients had acute MCA infarction due to occlusion of M1 segment of the clinically relevant MCA and were evaluated by DWI and a cerebral angiography.

Among them, 19 patients were excluded because they received thrombolytic

treatment (n=11), or had undetermined (n=12) or other determined causes (n=2) by TOAST classification. Finally 22 patients were enrolled for the data analysis. Fourteen of them belonged to LAA and 8 to CE. Hypertension was more frequently found in LAA than CE (P<0.05). There was no difference between the two groups in the other demographic factors (Table 1).

Table 1. Demographic characteristics of patients

	LAA (n = 14)	CE (n = 8)	*P* value
Age, year[1]	68±10	59±14	0.92
Sex, men	9 (64)	4 (50)	0.66
Hypertension	12 (86)	2 (25)	0.01
Diabetes mellitus	3 (21)	0 (0)	0.27
Smoking	7 (50)	4 (50)	1.00
Cardiac embolic sources	0 (0)	8 (100)	<0.01
Previous stroke	4 (29)	3 (38)	1.00

Numbers in parentheses are percentages.

[1] The values of age are means ± standard deviation.

LAA, large artery atherosclerosis; CE, cardioembolism.

B. Neurological deficits

The NIHSS score on admission was lower in LAA than in CE (6.86±3.74 versus 14.63±6.55, P<0.01, Figure 2A). The time interval from onset of stoke to

DWI was 51.14 hours in LAA and 44.25 hours in CE (*P*=0.71), and the interval from onset of stoke to angiographic studies was 66.71 hours in LAA and 53.25 hours in CE (*P*=0.40). The infarction volume was smaller in LAA than in CE (28.28±33.24 cm^3 versus 70.90±50.88 cm^3, Figure 2B). The infarction patterns on DWI – territorial infarction, defined as infarction involving one MCA segment distal to the MCA bifurcation or more, and subcortical infarction – were different between the groups (*P*=0.03, Figure 3, Table 2).

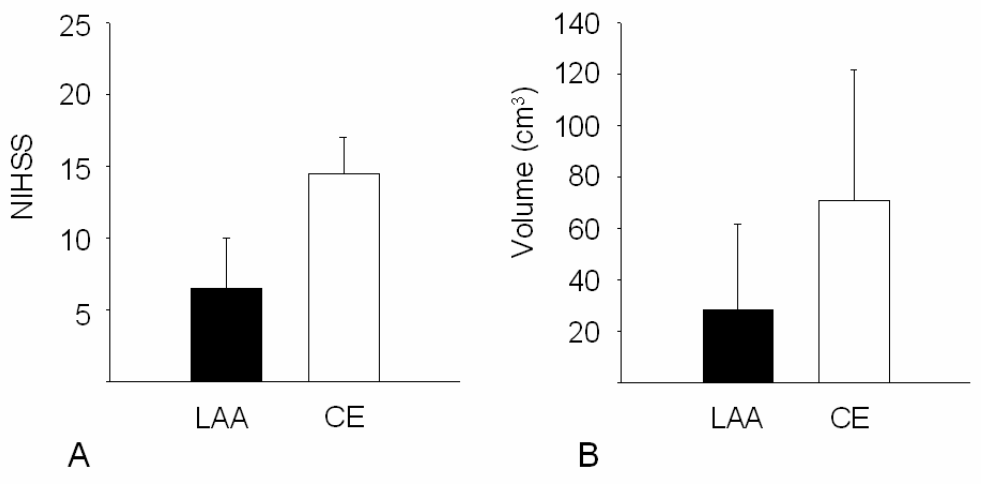

Figure 2. Comparison of stroke severity between the two patient groups. The National Institute of Health Stroke Scale (NIHSS) score on admission was lower in LAA than in CE (*P*<0.01, A). The infarction volume was smaller in LAA than in CE (*P*=0.03, B). LAA, large artery atherosclerosis; CE, cardioembolism.

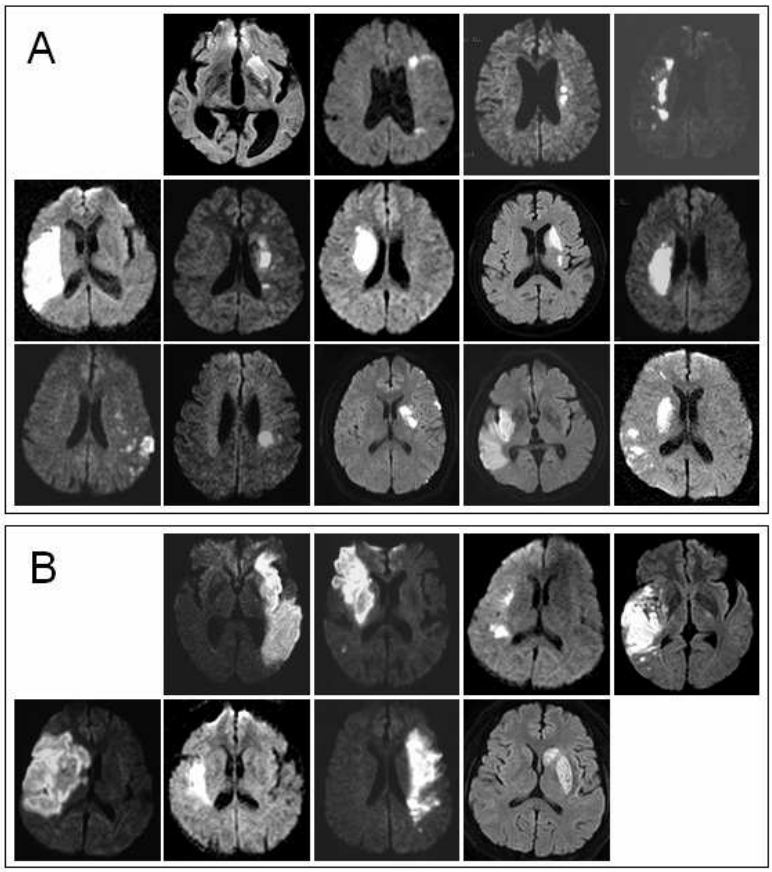

Figure 3. Representative diffusion weighted images of the patients. The infarction volume was smaller in LAA (A) than CE (B).

Table 2. Infarction patterns on diffusion weighted images

	LAA	CE	*P* value
Territorial infarction	2 (14)	5 (63)	0.03
Subcortical infarction	12 (86)	3 (37)	

Numbers in parentheses are percentages.

2. The effect of chronic cerebral hypoperfusion in the rat on stroke severity and arteriogenesis

Four of the 30 rats with BCAL died while all 26 rats with the sham operation survived. Rats with BCAL were subjected to MCAO/R (BCAL-MCAO, 20 rats) or sham operation for MCAO (BCAL-Control, 6 rats). Those with sham operation were also subjected to MCAO (Sham-MCAO, 21 rats) or sham operation (Sham-Control, 5 rats). Thirteen rats in Sham-MCAO and 10 in BCAL-MCAO were used for measurements of the infarction volume. And the others were used for investigating the presence of arteriogenesis.

A. Neurological deficits

Rats with BACL had higher rCP after MCAO than those with sham operation ($P<0.01$, Table 3). The other physiologic variables, except MAP, were not different between pre- and post-MCAO and between Sham-MCAO and BCAL-MCAO (Table 3). In Sham-MCAO, the MAP before MCAO was lower than that after MCAO ($P=0.02$, Table 3). The infarction pattern of BCAL-MCAO tended to be subcortical and that of Sham-MCAO to be territorial (Figure 4).

Table 3. Physiological variables

Group	Rectal temperature (℃)	MAP (mmHg)	$_{P}$H	Pco_2 (mmHg)	Po_2 (mmHg)	Hb (g/dL)	$_{R}$CP (%)
Sham-MCAO							
Before MCAO	37.0±0.1	92.5±2.2*	7.42±0.02	43.7±5.0	116.2±24.2	14.3±1.4	100.0±0.9
After MCAO	37.1±0.2	95.8±1.8	7.43±0.03	45.5±4.3	118.7±13.9	14.3±0.9	24.6±16.2†
BCAL-MCAO							
Before BCAL	37.0±0.1	97.0±7.4	7.43±0.02	44.8±4.1	137.2±8.6	13.3±1.0	
After BCAL	37.2±0.2	103.7±7.3	7.43±0.03	43.3±5.1	131.2±9.2	12.9±0.7	35.4±18.1
Before MCAO	36.9±0.3	97.8±4.6	7.43±0.05	42.8±4.0	128.8±9.4	11.9±2.0	102.2±14.9
After MCAO	37.1±0.4	103.8±6.5	7.43±0.02	43.5±5.2	123.5±12.0	12.9±0.8	46.9±17.7

Values are means ± standard deviation.

* P=0.02 different form that of Sham-MCAO after MCAO.

† P<0.01 different from that of BACL-MCAO after MCAO.

BCAL, bilateral common carotid artery ligation; MCAO, middle cerebral artery occlusion; Sham-MCAO, sham operated rats for BCAL, and then MCAO/reperfusion (MCAO/R); BCAL-MCA, rats that underwent BCAL, and then MCAO/R; MAP, mean arterial pressure; Hb, hemogrobin; $_{R}$CP, regional cerebral perfusion.

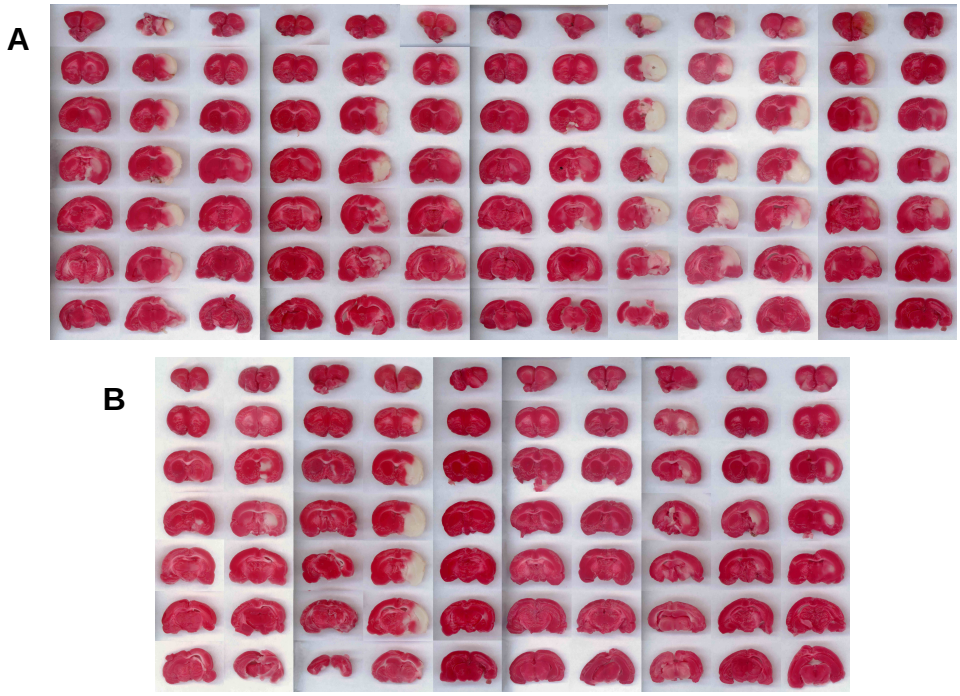

Figure 4. Brain slices stained with 2,3,5-triphenyltetrazolium chloride solution. The infarction pattern of Sham-MCAO tended to be territorial (A) and that of BCAL-MCAO to be subcortical (B).

The infarction volume of BACL-MCAO was smaller than that of Sham-MCAO (5.86±7.51% versus 20.31±12.00%, *P*=0.04, Figure 5A). The score of the motor disability scale was higher in Sham-MCAO than in BCAL-MCAO (3.08±0.59 versus 2.20±0.79, *P*=0.02, Figure 5B).

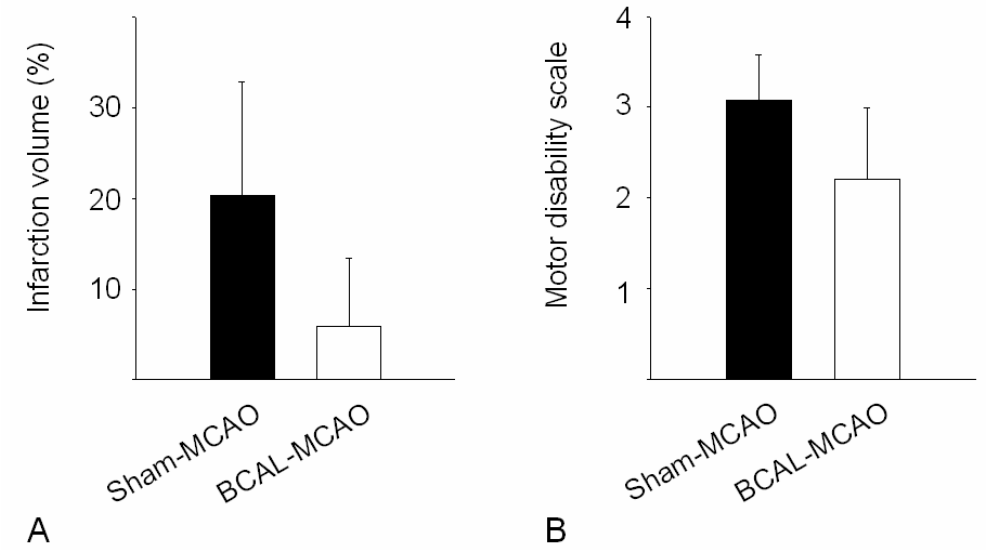

Figure 5. Comparison of the stroke severity between the two animal groups. Infarction volume of BACL-MCAO was smaller than that of Sham-MCAO (*P*=0.04, A). The motor disability scale score was higher in Sham-MCAO than in BCAL-MCAO (*P*=0.02, B).

B. Evaluation of arteriogenesis

Diameters of the bilateral PcomA, the bilateral PCA, and the BA in BCAL-Control or BCAL-MCAO were larger than those in Sham-Control or Sham-MCAO (*P*<0.01, Table 4, Figure 6-8). Diameters of the bilateral MCA were not different among the groups (*P*>0.05, Table 4, Figure 6, 8). The VT of the BA in

BCAL-Control or BCAL-MCAO were greater than those in Sham-Control or Sham-MCAO (1.12±0.07 and 1.08±0.01 versus 1.01±0.005 and 1.02±0.01, P<0.01, Figure 9). The increase in the VT reflects that the BA was lengthened by about 8-12% after BCAL. The VD in brain sections was increased in rats with BCAL when compared with those with sham operation.

Table 4. Vessel diameters

Vessel	Sham-Control	Sham-MCAO	BCAL-Control	BCAL-MCAO	*P* value
Right MCA	250.73±33.10	258.72±54.89	295.30±21.67	300.79±44.96	0.06
Left MCA	268.29±21.04	264.92±39.24	287.93±37.24	301.16±57.42	0.35
Right PcomA	201.88±23.82	221.04±76.60	456.30±29.26	385.87±77.98	<0.01
Left PcomA	229.52±48.12	248.80±37.25	497.45±103.52	413.42±51.53	<0.01
Right P1	221.14±38.41	207.17±32.84	411.29±100.52	435.57±51.89	<0.01
Left P1	204.99±23.69	209.40±34.89	426.88±48.38	412.21±61.91	<0.01
Right P2	235.00±33.17	231.67±42.62	355.00±40.37	325.00±88.35	<0.01
Left P2	222.50±50.58	260.00±21.91	370.00±103.73	341.00±67.90	<0.01
BA	373.75±15.69	329.58±57.52	448.10±60.84	484.59±50.78	<0.01

Values are means ± standard deviation (um).

MCA, middle cerebral artery; PcomA, posterior communicating artery; P1, precommunicating segment of the posterior cerebral artery (PCA); P2, postcommunicating segment of the PCA; BA, basilar artery.

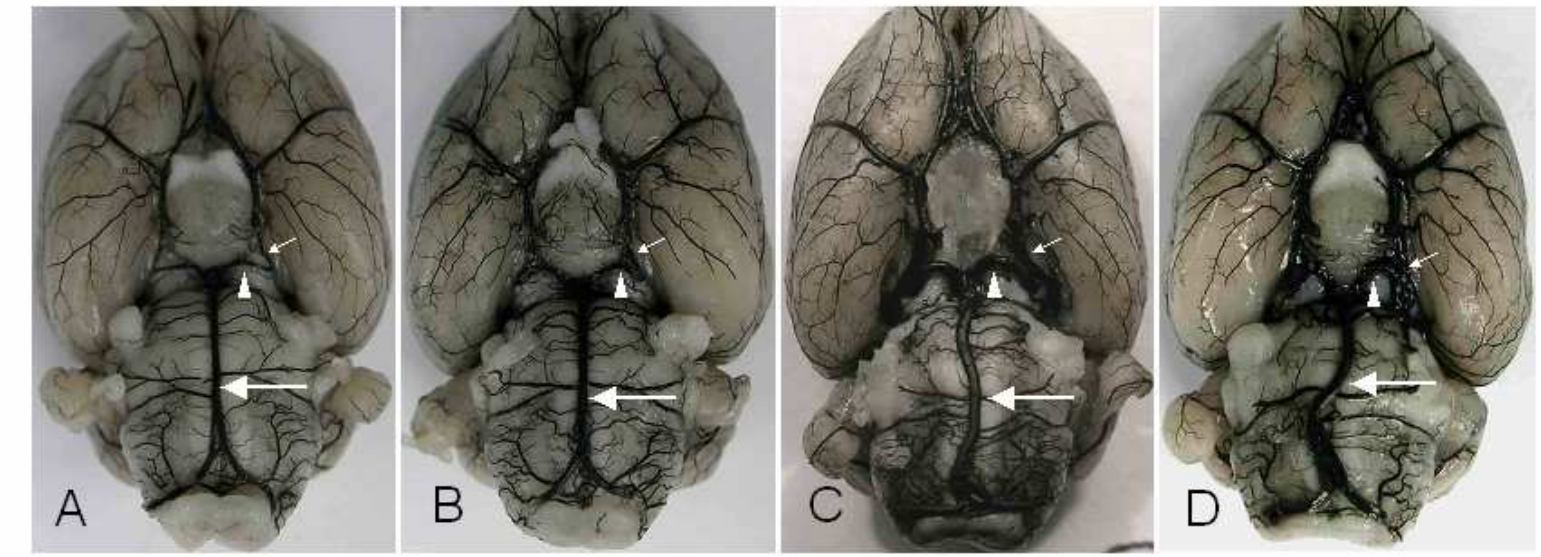

Figure 6. Representative photographs showing surface arteries after angiographies using latex-perfusion method. Diameters of the posterior communicating artery (small arrows), the posterior cerebral artery (arrowheads), and the basilar artery (Large arrows) appeared lager in the BCAL groups (C and D) than in the Sham groups (A and B). Sham-Control, sham operated rats both bilateral common carotid artery ligation (BCAL) and middle cerebral artery occlusion/reperfusion (MCAO/R); BCAL-Control, rats that underwent BCAL, and then sham operation for MCAO/R.

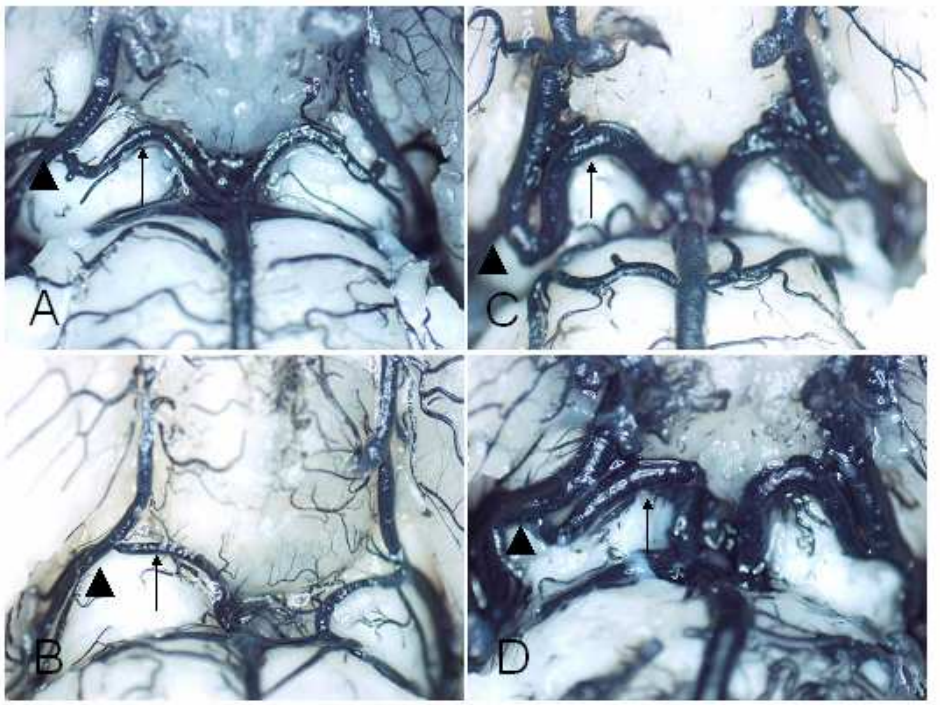

Figure 7. Representative photographs showing precommunicating and postcommunicating segments of the posterior cerebral artery (PCA). For the measurement of postcommunicating segments of the PCAs, the extreme portion of the medial temporal lobe was removed. Diameters of the bilateral precommunicating (small arrows) and postcommunicating segments (arrowheads) were larger in the BCAL groups (C and D) when compared with the Sham groups (A and B).

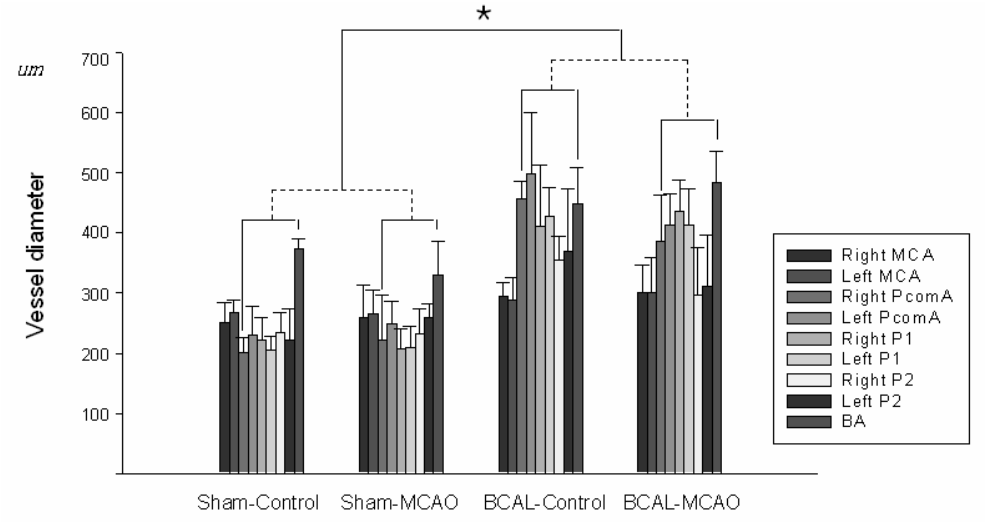

Figure 8. Vessel diameters of the surface brain arteries. Diameters of the posterior communicating artery (PcomA), the precommunicating (P1) and postcommunicating (P2) segments of the posterior cerebral artery, and the basilar artery (BA) in BCAL-Control and BCAL-MCAO were larger than those in Sham-Control and Sham-MCAO.

* $P<0.01$

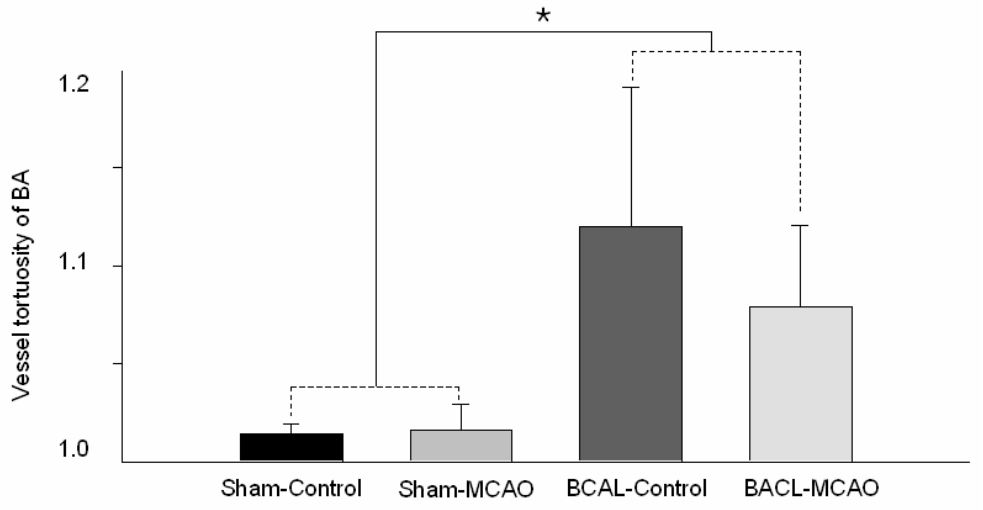

Figure 9. Vessel tortuosity of the basilar artery (BA). Vessel tortuosity of the BA was greater in the BCAL groups than in the Sham groups.

* $P<0.01$

The difference of the VD between the groups was greater in the sections closer to the occipital pole (5, 9, and 13 mm from the frontal pole). On the contrary, the VD was similar in the sections closer to the frontal pole (1 mm from the frontal pole, Table 5, Figure 10). The VD appeared to be increased in the capillary as well as in the arterial level. In the analysis of the VD according to the vessel diameter category, <10 um, 10-30 um, 30-70 um, 70-90 um, and >90 um, these differences were present (Table 5).

Table 5. Vessel density

Diameter (um)	VD (/mm^2) 1 mm from FP	VD (/mm^2) 5 mm from FP	VD (/mm^2) 9 mm from FP	VD (/mm^2) 13 mm from FP
Sham-Control				
<10	39.31±22.36	34.52±18.23	25.00±11.25	33.85±13.06
10-30	6.38±1.13	4.78±2.59	7.23±3.23	6.45±2.25
30-50	1.79±0.82	0.81±0.24	1.26±0.41	1.06±0.31
50-70	0.47±0.06	0.40±0.11	0.32±0.08	0.39±0.25
70-90	0.45±0.17	0.21±0.08	0.34±0.18	0.36±0.20
≥90	0.83±0.52	0.36±0.17	0.47±0.15	0.76±0.46
Total	49.22±21.81	41.07±19.80	34.61±12.12	42.87±15.10
Sham-MCAO				
<10	36.67±15.23	29.42±8.89	33.61±11.24	41.21±9.32
10-30	3.43±1.47*	3.46±2.09	3.89±1.90*	5.03±1.87
30-50	1.70±0.90	0.97±0.36	1.02±0.44	1.10±0.44
50-70	0.65±0.28	0.50±0.28	0.55±0.23	0.68±0.31
70-90	0.23±0.24	0.22±0.19	0.41±0.15	0.39±0.27
≥90	0.87±0.34	0.74±0.27*	0.75±0.35	1.21±0.56
Total	43.54±15.95	35.31±9.32	40.23±11.42	49.62±9.49
BCAL-Control				
<10	40.42±4.57	44.11±3.79	57.64±7.24†	67.54±9.41†
10-30	6.06±2.52	5.24±1.49	6.80±3.76	9.25±2.24
30-50	1.17±0.60	1.36±0.15†	1.34±0.57	1.85±0.70
50-70	1.10±0.38†	0.79±0.28†	0.62±0.33	0.96±0.11†
70-90	0.52±0.35	0.50±0.12†	0.67±0.12†	0.76±0.16
≥90	1.04±0.54	1.32±0.35†	1.46±0.14†	2.23±0.82
Total	50.31±6.76	53.32±5.21	68.53±11.66†	82.59±9.21†
BCAL-MCAO				
<10	42.27±25.58	48.33±3.76‡	58.15±17.39‡	52.73±19.97
10-30	6.45±2.34‡	5.23±3.10	6.16±2.81‡	7.42±2.44‡
30-50	1.78±0.46	1.17±0.27	1.50±0.47‡	1.44±0.46
50-70	0.93±0.50	0.79±0.12‡	0.80±0.34‡	0.84±0.40
70-90	0.53±0.29	0.46±0.18‡	0.59±0.30	0.48±0.26
≥90	1.61±0.92	1.14±0.22‡	1.12±0.18‡§	1.96±0.32‡
Total	53.58±26.19	57.12±3.85‡	68.33±17.71‡	64.88±21.20

Values are means ± standard deviation.

* $P<0.05$ different from Sham-Control.

† $P<0.05$ different from Sham-Control.

‡ $P<0.05$ different from Sham-MCAO.

§ $P<0.05$ different from BCAL-Control.

VD, vessel density; FP, frontal pole.

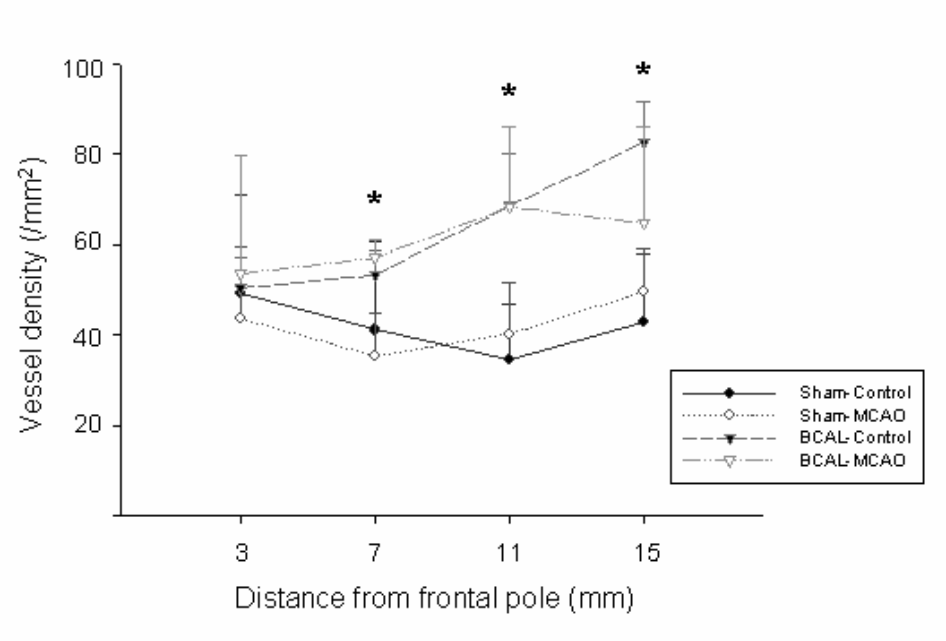

Figure 10. Comparison of the four animal groups in vessel density. The vessel density of brain sections at 5, 9, and 13 mm from the frontal pole in BCAL groups were larger than those in Sham groups.

* $P<0.05$

IV. DISCUSSION

Through this study, the hypothesis was tested that chronic cerebral hypoperfusion might be protective against subsequent severe ischemia, and it was proved that it induced adaptive growth of collateral circulation associated with a reduced infarct size.

Many clinical observations have shown that patients with atherothrombotic stroke had better neurological outcomes and smaller infarction volumes than those with cardioembolic stroke.[8, 9] However, the exact mechanism remains unknown. Although the size of thrombus or embolus and the location of the occluded vessels have been explanations for the difference of stroke severity between them, it has been doubtful whether they are really the matter, largely due to lack of a study for the comparison of them in the homogenous conditions.[8, 9, 26-28] In the first part of this study, homogenous patients, in terms of the site of arterial occlusion, were selected and divided into 2 groups, which were presumably caused by cardiac embolism and artery to artery embolism or in-situ thrombosis of the MCA. The latter group had significant stenosis of the tandem proximal artery that might have caused previous hypoperfusion. By the comparison of these rather homogenous groups, we demonstrated that the location of the occluded vessels was not the major decisive factor of the reduced neurological outcomes and infarction volumes in the patients with

atherothrombotic stroke. These findings were further examined in the experimental conditions using BCAL and MCAO models in the SD rats. Four weeks after BCAL or sham operation, the rats with BCAL had less severe neurological deficits than those with sham operation when the MCA was occluded, which confirmed findings in human stroke.

The growth of collateral circulation has been one of the explanations for the less severe stroke in patients with atherothrombotic stroke or with severe large arterial stenosis. However, the presence and difference of collateral circulation have not been systematically examined or compared between those patients because of the difficulty in assessment of the collateral circulation with a quantitative manner and the lack of the standard method to determine the magnitude of collateral circulations. Although studies in experimental animals may be enable to directly demonstrate the presence of collateral circulations and the quantitative assessment of them, there have been few studies for the examination of vascular responses in chronic hypoperfused state.[14-16] Patients with atherothrombotic stroke tend to have long standing severe stenosis of intracranial or extracranial cerebral arteries, which subsequently causes the brain region in a hypoperfusion state. From our previous studies, we demonstrated that chronic cerebral hypoperfusion induced tolerance to subsequent severe ischemic insult. Chronic hypoperfusion may also induce adaptive growth of collateral circulation, namely, arteriogenesis and/or

angiogenesis.[10-13] In the second part of experiments, vascular remodeling after BCAL was examined. According to the previous reports and our previous study using spontaneous hypertensive rats,[19, 20, 29] rCP was declined shortly after BCAL, but 4 weeks after BCAL, it was restored to the baseline level. Although rCP was not different between rats with BCAL and sham operation before MCAO, reduction of rCP induced by MCAO and measured from the outer surface of the cortex was significantly decreased in BCAL group, which indicated increase in local vascular reserve in rats with BCAL. This could be ascribed to the BCAL-induced arteriogenesis and angiogenesis as it was shown in this study that demonstrated increase of the vascular diameter and length on the brain surface and increase of vascular density in the cross-sectioned brain parenchyma. Increased vascular reserve could have saved some of the brain tissues from severe ischemic insults by MCAO.

Angiographies by use of latex perfusion method showed that the rats with BCAL had larger diameters of the PCA, the PcomA, and the BA than the sham-operated rats. The presence of arteriogenesis has been demonstrated in several chronic ischemic models such as ischemic hind-limb and chronic myocardial ischmia models.[13, 30] In the cerebral vasculature, Coyle and colleagues demonstrated that MCAO resulted in the increased tortuostiy of the vessels distal to the occluded MCA on the dorsal surface.[24] Enlargement of the ipsilateral PCA diameters was also shown in chronic 3-vessel (one carotid plus

both vertebral arteries) occlusion.[14, 15] Through this study, it was added to the previous reports that adaptive vascular remodeling occurred not only in the way of enlarging the diameter but also in the way of increasing the length of the large artery shown by increasing the VT of the BA.

This study demonstrated evidence of the adaptive vascular growth in all sizes of vessels assessed, namely, capillary and arteriole/arterial levels. Although occurrence of angiogenesis as well as arteriogenesis was feasible by chronic cerebral hypoperfusion,[31] it was uncertain, from the present study, whether the increase of VD was due to the increase in the absolute number of vessels or simply reflected the increased tortuosity. The increase of VD was significant at the posterior brain areas but not at the site close to the frontal pole. This could be expected because the PCA, which probably was the major collateral route in this model, supplies the posterior part of the brain.

V. CONCLUSION

By this study, the hypothesis was verified that chronic cerebral hypoperfusion might be protective against subsequent severe ischemia and adaptive vascular growth might play a role. To do this, data of stroke patients with MCA occlusion either from atherothrombotic or cardioembolic stroke were analyzed, and stroke severity and the presence of arteriogenesis were investigated in rats that were subjected to BCAL for 4 weeks and then MCAO.

1. The patients with MCAO from atherothrombotic stroke associated with significant stenosis of the tandem ICA had less severe neurological deficits and smaller infarction volumes than those with cardioembolic stroke.
2. The rats with BCAL for 4 weeks had less severe neurological deficits and smaller infarction volumes than the sham-operated rats when they were subjected to MCAO.
3. The rats with BCAL had the larger diameters of the PCA, the PcomA and the BA, and the longer length of the BA than the sham-operated rats, which indicated arterigenesis induced by BCAL.
4. VD, which was measured in the coronally cross-sectioned brain slices, was increased in the brain areas with BCAL that are supplied by the

PCA. Increased VD was observed in all sizes of vessels, which indicated potential development of angiogenesis as well as arteriogenesis in this model.

From these results, we concluded that chronic cerebral hypoperfusion induced the arteriogenesis and this might contribute to reduced stroke severity.

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국문요약

만성 뇌혈류 저하가 뇌졸중 중증도와 신생동맥형성에 미치는 영향

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김 서 현

동맥경화에 의한 뇌졸중은 심인성 뇌졸중에 비하여 크기가 작고 신경학적 결손이 덜한 것으로 알려져 있다. 죽상동맥경화에 의하여 뇌졸중이 발생한 환자는 폐색된 혈관의 기시부에 심한 협착이 동반되어 있을 확률이 높은데, 이러한 협착이 뇌졸중이 발생하기 전에 만성 뇌혈류 저하를 일으킬 수 있다. 만성 뇌혈류 저하는 신생동맥형성(arteriogenesis)을 유도하여 허혈에 의한 손상을 줄일 수 있다. 이러한 가설을 입증하기 위하여 중대뇌동맥 폐색에 의하여 뇌졸중이 발생한 환자를 동측 내경동맥에 심한 협착이 있는 환자와 이러한 이상 없이 심인성 색전 원인이 있던 환자의 두 군으로 나누어 뇌경색의 크기와 초기 신경학적 결손을 측정하여 비교하였다. 그

결과, 심한 협착이 있는 환자군이 뇌경색 크기가 작았으며 (P=0.03) 신경학적 결손이 경미하였다 (P<0.01). 두 번째 연구에서는 Sprague-Dawley 쥐에서 양측 총경동맥을 결찰함으로서 만성 뇌혈류 저하를 유도하였다. 총경동맥을 4 주 동안 결찰한 쥐와 그렇지 않은 쥐의 두 군을 대상으로 나일론 봉합 모델을 이용한 중대뇌동맥 폐색과 재관류를 시행하였다. 2,3,5-triphenyltetrazolium chloride 염색을 이용하여 뇌경색 크기를 측정한 결과, 양측 총경동맥 결찰을 한 쥐군에서 뇌경색 크기가 작았다 (P=0.04). 신생동맥형성 여부는 라텍스 관류법을 이용한 사후 뇌혈관조영술에 의하여 측정하였다. 양측 총경동맥 결찰을 한 쥐군이 결찰하지 않은 쥐군 보다 양측 후대뇌동맥과 양측 후교통동맥, 그리고 기저동맥의 지름이 더 컸으며, 기저동맥의 길이가 길었다 (P<0.01). 뇌 관상면의 혈관 밀도 역시 양측 총경동맥 결찰을 한 쥐군이 그렇지 않은 쥐군 보다 컸다 (P<0.05). 본 실험에서 만성 뇌혈류 저하 상태로 사전에 조작된 뇌 영역에 뇌졸중이 발생되면 그 손상은 적은데, 신생동맥형성이 이에 관계될 수 있다는 결론을 내릴 수 있었다.

핵심되는 말 : 만성 뇌혈류 저하, 중대뇌동맥 폐색, 급성 뇌경색, 신생동맥형성