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Embolic Cerebral Infarction Caused by Intraluminal Thrombus in the Carotid Siphon Successfully Treated With Combination of Anticoagulant and Antiplatelet Drugs

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A 54-year-old-man experienced serial ischemic embolic strokes and retinal artery embolism in the left carotid territory. In the acute phase, intraluminal thrombus in the left carotid siphon and frequent microembolic signals (MES) in the left middle cerebral artery were detected with magnetic resonance angiography (MRA) and transcranial Doppler (TCD). The patient was initially treated with only heparin for 3 days; however, more than 30 MES per 30 min were still detected. After adding ticlopidine as an antiplatelet therapy, MES were suppressed completely. After starting combination therapy of heparin (later warfarin) and ticlopidine, repeated MRA confirmed resolution of carotid thrombus and ischemic stroke did not recur. For management of intraluminal thrombus in the carotid artery, MES with TCD was useful in evaluating the risk of distal embolism. Combination treatment with anticoagulants and ticlopidine can both resolve the thrombus and prevent distal embolism. (*Circ J* 2005; **69:** 1147–1149)

Key Words: Anticoagulants; Antiplatelet therapy; Cerebral embolism; Intraluminal thrombus; Trancranial Doppler

Intraluminal thrombus in the carotid artery is identified in less than 3% of patients who undergo cerebral angiography for investigation of ischemic cerebrovascular symptoms[1,2] Carotid thrombi are usually associated with atherosclerotic stenotic lesions in the carotid bifurcation. To prevent recurrent stroke in these patients, the inhibition of distal embolism must be successful in addition to the resolution of intraluminal thrombus and surgical manipulation for stenotic lesion. The risk of distal embolism can be evaluated as a microembolic signals (MES) in transcranial Doppler (TCD) sonography[3] Previous studies recommended anticoagulation for the resolution of intraluminal thombus, however, the significance of antiplatelet drugs has been rarely evaluated[4]

We report a patient with recurrent ischemic stroke and retinal artery embolism in whom intraluminal thrombus in the carotid siphon and frequent MES in the middle cerebral artery (MCA) were successfully resolved with combination therapy of anticoagulant and antiplatelet drugs.

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Case Report

A 54-year-old man experienced right-hand numbness and difficulties with reading and writing in May 2000. A brain computed tomography revealed a low-density area with partial high density in the left parieto-temporal lobe. On admission to hospital in June, the patient's physical examination was normal and neurological examination revealed right-lower quadrantanopsia, slight right hemiparesis and sensory aphasia.

Three weeks later, the patient suddenly developed a loss of vision in the left eye and became confused. Funduscopic examinations revealed branch occlusion of the left retinal artery. Laboratory tests showed the following abnormalities: thrombin-antithrombin III complex, 4.48 μg/L (normal range, 0-3.0); D-dimer, 1.18 μg/ml (0-0.50); β-thromboglobulin, 473 ng/ml (<50); and platelet factor-4, 115 ng/ml (<20). Prothrombin time, activated partial thrombin time (APTT), protein S and C, anticardiolipin antibody and lupus anticoagulant were all within normal limits.

Diffusion-weighted magnetic resonance imaging (MRI) showed small hyperintensity regions scattered within the left MCA territory (Fig 1A). Magnetic resonance angiography (MRA) revealed intraluminal thrombus in the cavernous portion of the left internal carotid artery (Fig 1C). TCD monitoring detected 53 MES over 30 min in the left MCA. To determine the embolic source, the following examinations were performed and showed normal findings: electrocardiogram (ECG), Holter ECG and transthoracic echocardiography. Carotid ultrasonography showed no atheromatous lesion in the carotid bifurcation. In transesophageal echocardiography (TEE), thrombus in the left atrium or patent foramen ovale was not detected, but an

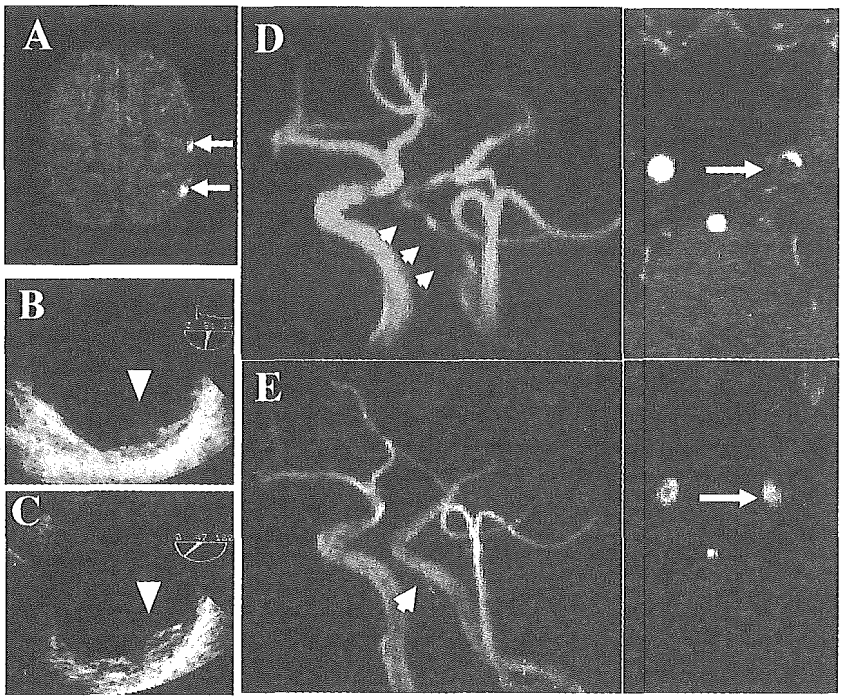

Fig 1. Diffusion weighted images (DWI) of the magnetic resonance imaging (A), transverse images of aortic arch obtained using transesophageal echocadgiography (TEE) (B,C), maximum-intensity projection images (Left) and source images (Right) of the magnetic resonance angiography (D, E). (A) DWI images showing scattered regions of hyperintensity in the left middle cerebral artery territory. (B) Atheromatous plaque with a mobile component (arrowhead) in the aortic arch on TEE. (C) A week after anticoagulation therapy, the mobile component has almost disappeared (arrowhead). (D) An intraluminal filling defect in the left carotid siphon can be seen (arrowheads). Round and concentric defects are typical of an intraluminal thrombus (arrow). (E) Resolution of the intraluminal thrombus (arrow and arrowhead) after combination therapy with anticoagulant and antiplatelet drugs.

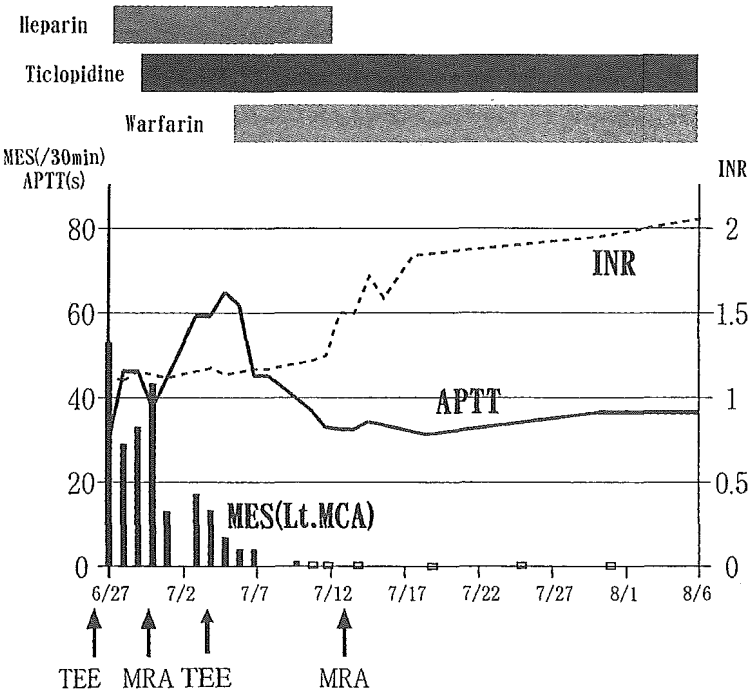

Fig 2. Time course of microembolic signals (MES) in transcranial Doppler with anticoagulation (heparin or warfarin) and antiplatelet therapy (ticlopidine). The number of MES in the left middle cerebral artery is shown as embolus per 30 min. The white box on the baseline indicates no embolic signal. Activated partial thrombin time (APTT) and international normalized ratio (INR) are shown as solid and dotted lines respectively. MCA, middle cerebral artery; TEE, transesophageal echocadgiography; MRA, magnetic resonance angiography.

atherosclerotic plaque protruding in the aortic arch with a mobile component was found (Fig 1B). We diagnosed cerebral and retinal embolism due to intraluminal thrombus in the carotid siphon. The potential cause of intraluminal thrombus was aortic complex lesions.

The patient was soon treated with heparin intravenously (12,000 U/day); however, TCD monitoring for MES showed more than 30 emboli in spite of effective anticoagulation treatment as judged with APTT (Fig 2). To prevent distal embolism, we started treatment with ticlopidine as an antiplatelet strategy in combination with anticoagulant. Thereafter, serial TCD monitoring showed a marked decrease of the MES in the left MCA. A week after starting anticoagulation therapy, repeated TEE showed resolution of mobile components in an atherosclerotic plaque (Fig 1C). We continued combination therapy of anticoaguilant and antiplatelet drugs after replacement of heparin by warfarin. Two weeks after starting combination therapy, repeated MRA revealed the resolution of intraluminal thrombus in the left carotid siphon (Fig 1D). The patient recovered consciousness within 1 week of starting treatment and the aphasia diminished by the end of July. He was discharged

for normal daily activities the next month.

Discussion

In the present case, we successfully treated high-risk patient with intraluminal thrombus in the carotid siphon using a combination of anticoagulant and antiplatelet therapy. In terms of diagnosis, we first had to rule out the possibility of carotid dissection. Carotid dissection was highly unlikely in the present case based on the following findings: although the typical indicator of dissection on MRI is a narrowed lumen surrounded by crescent and eccentric hyperintense signal, in the present case the filling defect was round and concentric (Fig 1D, arrow).[5] Dissection usually terminates before the petrous portion and in the present case the filling defect was seen at the carotid siphon. Furthermore, the present case had multiple ischemic events, although the recurrence of dissections in the same artery is rare.

Intraluminal thrombus is usually associated with an advanced atherosclerotic lesion in the carotid bifurcation![1,2,6] Cardiac embolic sources may also cause an intraluminal carotid thrombus. In the present case, we failed to detect an atherosclerotic lesion in the carotid bifurcation or another embolic source including: the left atrial thrombus, patent foramen ovale or paroxysmal atrial fibrillation. Other possible causes of hypercoagulability were not found, aside from an activated platelet function[7] Therefore, an atheromatous plaque with a mobile component in the aortic arch could be the only embolic source in this patient. Several papers have reported TEE recognition of aortic atheroma as the potential source of embolic stroke![8,9]

The novel finding in the present case was the possibility that ticlopidine suppressed MES from an intraluminal thrombus. We used ticlopidine in expectation of a stronger antiplatelet effect than aspirin because a randomized trial in high-risk patients demonstrated that ticlopidine was somewhat more effective than aspirin in preventing stroke![10] The detection of MES is potentially useful for identifying acute stroke patients at high risk of recurrent stroke or evaluating the effectiveness of anticoagulant or antiplatelet therapies in these patients![11,12] In the present case, frequent MES were still detected after anticoagulant therapy but only suppressed after the administration of ticlopidine. There is no doubt that anticoagulants are effective in the resolution of carotid thrombus![2,4] However, the role of antiplatelet therapy has not been clearly demonstrated. Our findings suggest the importance of antiplatelet therapy for the suppression of distal embolism due to intraluminal thrombus. The patient received a combination of ticlopidine and warfarin for the prevention of recurrent stroke at discharge. The ongoing Aortic arch Related Cerebral Hazard study may determine whether or not antiplatelet drugs are more effective than anticoagulation alone![13] The present findings demonstrate the usefulness of TCD monitoring in the assessment of distal embolism in intraluminal thrombus and of the recommended combination of anticoagulant and antiplatelet drugs for the resolution of thrombus and prevention of recurrent stroke.

Acknowledgments

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Chronic Mild Reduction of Cerebral Perfusion Pressure Induces Ischemic Tolerance in Focal Cerebral Ischemia

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Background and Purpose—Neurons acquire tolerance to ischemic stress when preconditioning ischemia occurs a few days beforehand. We focused on collateral development after mild reduction of perfusion pressure to find an endogenous response of the vascular system that contributes to development of ischemic tolerance.

Methods—After attachment of a probe, the left common carotid artery (CCA) of C57BL/6 mice was occluded. The left middle cerebral artery (MCA) was subsequently occluded permanently on days 0, 1, 4, 14, and 28 (n=8 each). The change in cortical perfusion during MCA occlusion was recorded. A sham group of mice received only exposure of the CCA and MCA occlusion 14 days later. In apoE-knockout mice, the MCA was occluded 14 days after CCA occlusion or sham surgery. Infarct size and neurologic deficit were determined 4 days after MCA occlusion.

Results—Mice that had 45% to 65% of baseline perfusion after CCA occlusion were used. Cortical perfusion after MCA occlusion was significantly preserved in day 14 (47±16%) and day 28 (46±7%) groups compared with day 0 (28±8%), day 1 (33±19%), day 4 (29±16%), and sham groups (32±9%). Infarct size and neurologic deficits were also attenuated in day 14 and day 28 groups compared with other groups. In apoE-knockout mice, there was no significant difference in perfusion, neurologic deficits, or infarction size between groups with and without CCA occlusion.

Conclusion—Chronic mild reduction of perfusion pressure resulted in preservation of cortical perfusion and attenuation of infarct size after MCA occlusion. These responses of collaterals were impaired in apoE-knockout mice. (***Stroke.* 2005;36:2270-2274.**)

Key Words: chronic ischemia ■ chronic perfusion ■ collateral circulation ■ focal ischemia ■ ischemic tolerance

Collateral circulation through leptomeningeal vessels may determine the severity of ischemic injury after occlusion of middle cerebral artery (MCA) in patients with stroke.[1] Although chronic hypoperfusion is believed to be critical for collateral development,[2] factors leading to it are uncertain. For better understanding of collateral circulation in cerebral ischemia, an animal model in which cerebral collaterals can be investigated is needed. Recently, Busch et al[3] demonstrated that 3-vessel (one carotid plus both vertebral arteries) occlusion induced arteriogenesis at the circle of Willis in hypoperfused rat brain. They found that the diameter of the posterior cerebral artery enlarged significantly 1 week after 3-vessel occlusion,[3] but the distal leptomeningeal collaterals that determine stroke outcome did not change. Although morphologic assessment of leptomeningeal collaterals requires multivessel angiography[4] or latex perfusion methods,[5] the level of collateral circulation after cerebral ischemia can be functionally examined by measuring the change of cerebral perfusion at the time of MCA occlusion. In this study, we examined the effect of a mild reduction of cerebral perfusion pressure by occlusion of the left common carotid artery (CCA) on collateral development and infarct size after MCA occlusion. Furthermore, we examined the effect of apoE deficiency on development of collateral circulation after CCA occlusion because arteriogenesis in knockout mice is impaired in a hindlimb ischemia model.[6]

Materials and Methods

C57BL/6 strain mice were obtained from Charles River (Yokohama, Kanagawa, Japan). apoE-knockout mice, originally produced by Zhang et al,[7] were purchased from the Jackson Laboratory (Bar Harbor, Maine). All mice used in this study were mature males aged 12 to 16 weeks. Mice were given free access to food and water before surgery. The experimental procedures involving laboratory animals have been approved by the Institutional Animal Care and Use Committee of the Osaka University Graduate School of Medicine.

Surgery

General anesthesia was induced with 4.0% halothane and maintained with 0.5% halothane with an open facemask. A polyacrylamide column with an inner diameter of 0.8 mm for measurement of cortical microperfusion by laser-Doppler flowmetry (Advance laser Flowmetry, model ALF-21; Advance Co) was attached with dental

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cement to the intact skull 3.5mm lateral to the bregma. Laser Doppler flowmetry is not quantitative, but provides a reliable estimate of relative cerebral blood flow. The residual zero flow signal at the time of killing was <2% of baseline perfusion level in a preliminary experiment (n=10). Therefore, we did not determine it in each mouse in the following experiments. Body temperature was monitored with a rectal thermometer and maintained at 37.0°C with a heat lamp.

Common Carotid Artery Occlusion

In the first experiment, in each of 50 C57BL/6 mice, the CCA was ligated and the change in cortical perfusion after CCA occlusion was recorded. Seven days later, mice were killed with an overdose of pentobarbital. The brains were removed, fixed by immersion in an alcohol-5% acetic acid solution for 5 hours at 4°C, dehydrated, and embedded in paraffin. Tissue sections (5 μm) were obtained every 1mm, beginning at the frontal pole, and were examined after conventional staining with hematoxylin and eosin (H&E). In the sections including the hippocampus, the terminal deoxynucleotidyl-transferase (TdT)-mediated dUTP-biotin nick end-labeling (TUNEL) procedure was performed using the Apoptag in situ Detection Kit (Chemicon). In another 8 mice that had 45% to 65% of baseline perfusion after CCA occlusion, the cortical perfusion recordings were measured at 2 hours and 1, 4, 14, and 28 days after CCA occlusion. The change of cortical perfusion after sham surgery was also recorded in the other 8 mice. The level of cortical perfusion was expressed as a percentage of the baseline value.

Middle Cerebral Artery Occlusion Subsequent to Common Carotid Artery Occlusion

In the second experiment, the CCA was ligated under halothane anesthesia. In mice that had 45% to 65% of baseline cortical perfusion after CCA occlusion, the MCA was occluded using electrocoagulation as described previously[8] on days 0, 1, 4, 14, and 28 (n=8 each). Sham-group mice (n=8) received only exposure of CCA and MCA occlusion 14 days later. Mice were placed in the recumbent position, and a vertical skin incision was made at the midpoint between the left orbit and the external auditory canal. The mandible was pulled downward to expose the skull base. A small burr hole was made in the skull over the left MCA. The MCA was permanently occluded with a microbipolar electrocoagulator just proximal to the point where the olfactory branch came off. Cortical perfusion was monitored under halothane anesthesia for 15 minutes after MCA occlusion. Four days after MCA occlusion, mice were evaluated for neurologic deficits by a blind observer. The neurologic deficit score assignment of 0 to 4 was based on methods described previously by Yang et al[9]: 0, no neurologic deficits; 1, failed to extend right forepaw while held by the tail; 2, circled to the right; 3, fell to the right; or 4, unable to walk spontaneously. Then, mice were killed under pentobarbital anesthesia, and their brains were removed, fixed, and embedded in paraffin. The volume of infarction was measured using MCID Image Analysis Software (Imaging Research). The volume (mm^3) was determined by integrating the appropriate area and the section thickness. To visualize the capillary perfusion, we labeled plasma with dichrolotriazinyl amino fluorescein (DTAF; excitation 489 nm, emission 515 nm; Sigma-Aldrich) as described previously.[8] After MCA occlusion for 6 hours in mice with CCA occlusion or sham operation 14 days earlier, 50 μL of DTAF conjugated to mouse serum was injected for 10 seconds into the saphenous vein. Thirty seconds after completion of the injection, each mouse was decapitated and the brains were fixed in 80% ethanol for 24 hours. Brain slices, 50 μm in thickness, were prepared with a vibratome and examined under a fluorescence microscope. In mice with CCA occlusion or sham operation 14 days earlier (n=5 each), a femoral artery was cannulated with a PE-10 polyethylene tube at the time of MCA occlusion. Blood pH, Pa_{O_2}, and Pa_{CO_2} were measured using the Acid-Base Laboratory system (ABL550; Radiometer).

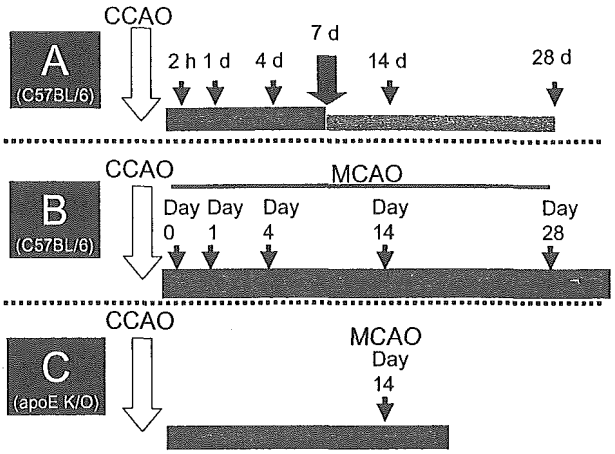

Figure 1. Experimental schedule. (A) The left CCA in 50 C57BL/6 mice was occluded and their brains were examined 7 days later. In another 16 mice, the cortical perfusion recordings were monitored until 28 days after CCA occlusion (n=8) or sham operation (n=8). (B) The left CCA was ligated first, and the left MCA was subsequently occluded on days 0, 1, 4, 14, and 28 (n=8 each). Neurologic deficit and infarct size were evaluated 4 days after MCA occlusion. (C) In apoE-knockout mice, the left MCA was permanently occluded 14 days after CCA ligation (n=8) or sham operation (n=8). CCAO indicates common carotid artery occlusion; MCAO, middle cerebral artery occlusion.

Effect of Common Carotid Artery Occlusion in apoE-Knockout Mice

In the last experiment, we used apoE-knockout mice. The left CCA of apoE-knockout mice (n=8) was ligated under halothane anesthesia. Fourteen days later, the left MCA was permanently occluded. Sham-group mice (n=8) received only exposure of the left CCA and occlusion of the left MCA 14 days later. Four days after MCA occlusion, mice were evaluated for neurologic deficits, and they were killed and their brains were examined for infarction size. The experiment schedule is shown in Figure 1.

Statistics

All values are presented as mean±standard deviation. One-way analysis of variance (ANOVA) was performed with Scheffe's multiple comparisons test to assess differences in perfusion change, neurologic deficit, score and infarct size between day 0, day 1, day 4, day 14, day 28, and sham groups. The perfusion values after CCA occlusion were analyzed by repeated-measures ANOVA followed by a post hoc Dunnett test. A Mann–Whitney U test was performed to assess differences between 2 groups of apoE-knockout mice. Pearson's test was used to evaluate the relationship between perfusion change after CCA occlusion and that after MCA occlusion. $P<0.05$ was considered significant. All statistical analyses were conducted using SPSS/Windows software, version 11.5J (SPSS Japan Inc).

Results

Unilateral occlusion of the left CCA resulted in 40% to 70% of baseline microperfusion over the MCA area in most mice. However, in 6 mice, cortical microperfusion was reduced to less than 35% of baseline (Figure 2). On the basis of histologic examination, one mouse showed infarction in the cortex, caudoputamen, and hippocampus, and 2 mice showed ischemic neuronal damage in the hippocampus with fragmented DNA detected by the TUNEL method (Figure 2B). In the other 47 mice, no ischemic damage was found (Figure 2B). Because more than 70% of mice had 45% to 65% of baseline cortical perfusion, we used those mice in the subse-

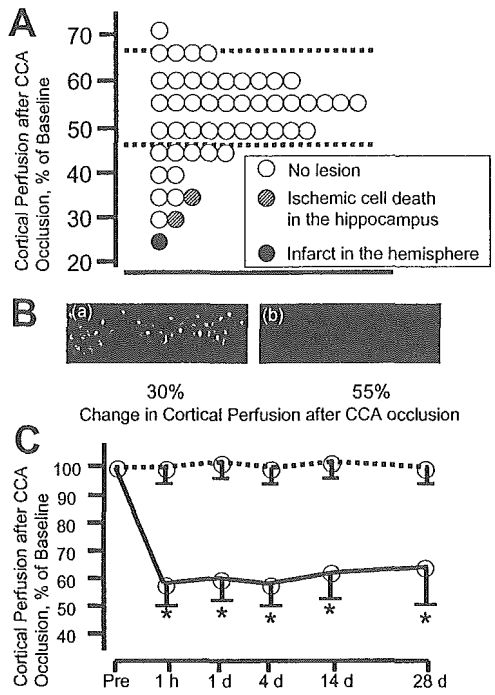

Figure 2. (A) Cortical perfusion as percent of baseline and histologic damage after CCA occlusion. Results from 50 C57BL/6 mice are plotted based on the change in perfusion and histologic damage. Three of 6 mice that showed less than 35% of baseline perfusion had ischemic neuronal damage or infarction in the ipsilateral hemisphere. Mice were used in subsequent experiments, which showed 45% to 65% of baseline perfusion after CCA occlusion (range indicated with dotted lines). (B) (a) TUNEL staining in the hippocampal CA1 sector of a mouse showing 30% of baseline perfusion after CCA occlusion. Numerous neurons with positive reaction were observed. (b) In the hippocampus of a mouse showing 55% of baseline perfusion, there was no TUNEL-positive neuron. (C) Cortical perfusion values after CCA occlusion (solid lines) and sham operation (dotted lines). *$P<0.01$ compared with the sham operation.

quent experiment. In the control group, the mean cortical perfusion after the sham operation varied from 90% to 111% without any significant changes between any time intervals. The cortical perfusion values decreased to 59.0±4.6%, 59.3±6.9%, and 55.4±9.0% at 2 hour and 1 and 4 days, respectively, and remained significantly lower at 14 (61.4±11.6%) and 28 days (63.2±14.0%) after CCA occlusion as compared with the baseline value (Figure 2C).

The change in the cortical perfusion after MCA occlusion compared with that before CCA operation, the perfusion change at the time of MCA occlusion, neurologic deficit score, and infarct size 4 days after MCA occlusion are shown for each group in Figure 3. CCA occlusion and subsequent MCA occlusion reduced perfusion to 15% to 20% in day 0, day 1, and day 4 groups (Figure 3A). However, in day 14 and day 28 groups, the change in perfusion after CCA and MCA occlusion were approximately 30% and were significantly higher than those of day 0, day 1, and day 4 groups (Figure 3A). In the sham-operation group, the change in perfusion after sham CCA operation and MCA occlusion was 31.8±9.0%, higher than those in day 0, day 1, and day 4 groups but not significant different compared with those in day 14 and day 28 groups (Figure 3A). At the time of MCA occlusion, cortical perfusion reduced to approximately 30% of baseline in day 0, day 1, day 4, and sham CCA exposure groups (Figure 3B). However, in day 14 and day 28 groups, the changes in perfusion at the time of MCA occlusion were 47.6±15.8% and 46.1±6.7%, respectively, and were significantly preserved compared with other groups (Figure 3B). In day 14 and day 28 groups (n=16), there was significantly negative correlation between the perfusion change after CCA occlusion and that at the time of MCA occlusion ($r=-0.686$, $P<0.01$). Both neurologic deficit score (Figure 3C) and infarction size (Figures 3D and 4) were significantly attenuated in day 14 and day 28 groups compared with other groups. Reduction of infarction size was markedly observed in the cerebral cortex (Figures 3D and 4). Capillary fluorescence was hardly observed in the center of the MCA territory in sham operation group (Figure 4). In contrast, capillary perfusion in the same territory was better preserved in day 14 group (Figure 4). The physiological variables were determined for mean arterial blood pressure (day 14 group, 76.6±4.3 mm Hg; sham operation group, 77.0±3.4 mm Hg), $Paco_2$ (day 14 group, 35.2±3.3 mm Hg; sham operation group, 34.4±2.8 mm Hg), Pao_2 (day 14 group, 119.6±9.2 mm Hg; sham operation group, 120.2±8.8 mm Hg), and pH (day 14 group, 7.29±0.07; sham operation group, 7.32±0.04 mm Hg). There were no significant differences in any of the variables between day 14 and sham-operation groups.

In apoE-knockout mice, the change in cortical perfusion, the neurologic score, and infarct size are shown in Figure 5. In apoE-knockout mice, the CCA occlusion treatment group (n=8) and the sham CCA exposure group (n=8) showed no significant difference in cortical microperfusion after CCA occlusion and subsequent MCA occlusion (29.5±9.0% compared with 30.4±8.5%), perfusion change at the time of MCA occlusion (36.4±11.8% compared with 30.2±7.8%), in neurologic scores (1.5±0.8 compared with 1.9±1.0), or in infarct size (31.5±8.2 mm^3 compared with 37.0±5.3 mm^3, $P=0.14$).

Discussion

Leptomeningeal collaterals that may maintain perfusion beyond the site of an arterial occlusion have been appreciated as an important factor both in modifying the size of infarction and in clinical outcome after embolic occlusion of the MCA.[1,10] Angiogenesis after MCA occlusion has been intensively investigated.[11,12] However, little is known about the factors that determine collateral development at the time of embolic occlusion. MCA occlusion has been shown to induce growth and enlargement of surface collaterals in the ischemic border.[13] Chronic hypoperfusion resulting from extracranial and intracranial occlusive disease promotes leptomeningeal collateral formation, although collaterals require time to develop.[5,10,13] The precise role of chronic hypoperfusion in collateral development remains unclear, although it is known that the efficacy of collateral vessels also depends on age, hypertension, and associated comorbidities.[14]

Recently, arteriogenesis at the circle of Willis has been demonstrated clearly in a 3-vessel (1 carotid plus both

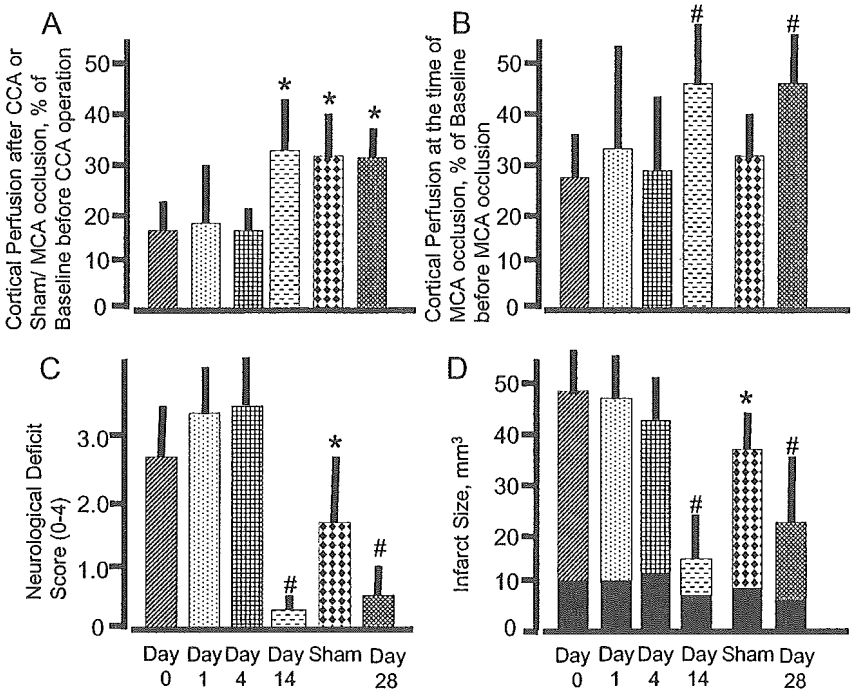

Figure 3. Effect of CCA occlusion and sham CCA operation (A) on the cortical perfusion after MCA occlusion compared with that before CCA operation, (B) on the cortical perfusion at the time of MCA occlusion compared with that before MCA occlusion (C) on the neurologic deficit score, and (D) on the infarct size. In (D), infarct size of the subcortical area in each group was shown as black parts of the columns. Day 0 indicates MCA occlusion on the same day as CCA occlusion. MCA was occluded on days 0, 1, 4, 14, and 28 after CCA occlusion. Sham group mice received only exposure of CCA 14 days before MCA occlusion. * $P<0.05$ compared with day 0, day 1, and day 4 groups. #$P<0.05$ compared with day 0, day 1, day 4, and sham groups. In day 14 and day 28 groups, cortical perfusion after MCA occlusion is preserved, neurologic scores are less severe, and infarct size is attenuated.

vertebral arteries) occlusion model.[3] Although reduction of perfusion pressure promotes arteriogenesis at the circle of Willis and in the distal anastomoses that have been described as "pre-existing collateral arterioles,"[15] the diameters of leptomeningeal anastomoses in the 3-vessel occlusion model did not change.[3] The estimation of vessel diameters may be insufficient for assessing collateral development, particularly in the ischemic condition. Resting cortical perfusion level remained 60% of baseline up to day 28 after CCA occlusion (Figure 2C), suggesting us to use a functional evaluation of collateral circulation by measuring the cortical perfusion at the time of MCA occlusion. We first examined the effect of unilateral CCA occlusion on cortical perfusion and histologic damage. Very few mice (6 of 50) showed moderate to severe ischemia (<35% of baseline) after unilateral CCA occlusion, and only 3 of them had histologic damage in the affected hemisphere. Therefore, in the present study, we used mice that showed 45% to 65% of baseline cortical perfusion.

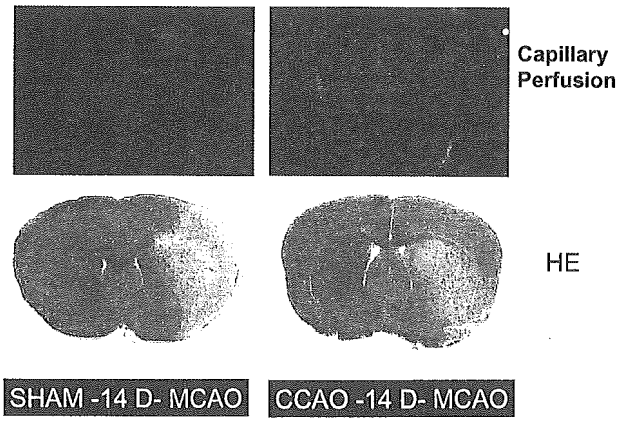

Figure 4. Capillary perfusion in the cerebral cortex and hematoxylin and eosin staining in the whole brain after MCA occlusion. In the mouse that received a sham CCA operation 14 days before MCA occlusion (SHAM-14D-MCAO), severe reduction of perfused microvessels and infarction was seen in the entire MCA territory. A preserved microcirculation and smaller infarct area was seen when CCA was occluded 14 days before MCA occlusion (CCAO-14D-MCAO).

Our results demonstrated that unilateral CCA occlusion treatment given 14 days before MCA occlusion preserved cortical perfusion and reduced infarct size markedly in the cerebral cortex after MCA occlusion. Negative correlation between changes in perfusion after the first CCA occlusion and at the time of subsequent MCA occlusion in day 14 and day 28 groups suggests that sufficient reduction of perfusion pressure is needed for collateral development. The time required to develop the effects in the present study is in agreement with the findings about collateral vessel development in hindlimb ischemia models.[16] In general, the development of preexisting collateral arterioles into large conductance vessels may take days to weeks after a critical stenosis of the proximal artery.[15] A direct neuroprotective preconditioning effect after chronic ischemia as shown in the previous study[17] may in part contribute to the effect in day 14 and day 28 groups. Furthermore, coagulation status such as platelet accumulation and fibrin deposition may change after chronic ischemia as suggested in hypoxia-tolerant states.[18]

Because several studies demonstrated that disordered lipid metabolism may impair collateral vessel growth and angiogenesis in other organs,[6] we examined the effect of unilateral CCA occlusion in apoE-knockout mice. We found that collateral development was impaired in apoE-knockout mice, suggesting that common mechanisms underlie arteriogenesis in the brain and systemic circulation. Based on the previous findings, possible mechanisms of collateral development after chronic hypoperfusion would be (1) fluid shear stress leading to endothelial activation, (2) monocyte invasion, and (3)

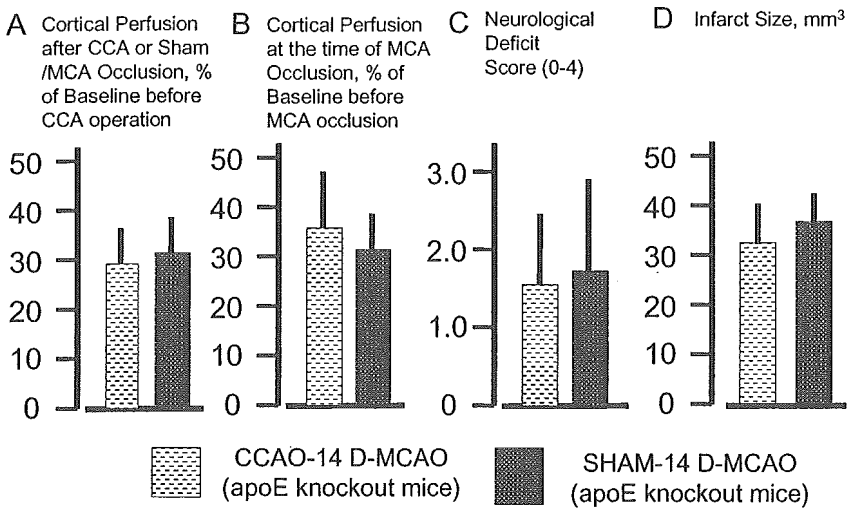

Figure 5. Diminished effect of CCA occlusion treatment in apoE-knockout mice. apoE-knockout mice received CCA occlusion or sham surgery; 14 days later, they received MCA occlusion. There is no significant difference in the (A) cortical perfusion after CCA and MCA occlusion, (B) cortical perfusion at the time of MCA occlusion, (C) neurologic deficit score, and (D) infarct size.

proliferation of smooth muscle cells and vessel enlargement.[19] Involvement of growth factors or cytokines such as VEGF[6] and granulocyte-macrophage colony-stimulating factor[20] in collateral development in the brain will need to be investigated further.

Endogenous adaptive responses to ischemic insults such as ischemic tolerance[21] have received much attention, and it is widely believed that this phenomenon is largely the result of an endogenous response such as gene expression in the preconditioned neuron.[22,23] However, vessel components should also be targets for investigation of endogenous response. Thus, we have clarified that chronic mild reduction of perfusion pressure leads to collateral development and brain protection after focal cerebral ischemia.

Acknowledgments

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5. Future Aspects of Gene Therapy in Acute Ischemic Stroke

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Key words: gene therapy, brain ischemia, macrophage, neurogenesis

Neural stem/progenitor cells remain in the adult mammalian brain, including the human brain. Neurogenesis continues throughout life in the two restricted zones, the hippocampal subgranular zone (SGZ) and the rostral migratory stream, where newly generated immature neurons migrate from the anterior subventricular zone (SVZ) into the olfactory bulb. Brain injury including ischemia stimulates neurogenesis in the SGZ and SVZ (1, 2). Therefore, therapeutic strategy for enhancing neurogenesis after ischemia may be of value for promoting functional recovery in stroke patients with neurological deficits. Intracerebral or intraventricular injections of neurotrophic factors could stimulate neurogenesis in the ischemic hippocampus and caudoputamen (3, 4). However, dependence on invasive surgical procedures for delivery could limit clinical application (Fig. 1A, B). Therefore, non-invasive, safe, and inexpensive strategies would be required for clinical application for enhancing neurogenesis in stroke patients. Several previous studies including our own have demonstrated that circulating monocytes or macrophages begin to infiltrate ischemic tissue after infarction develops (5). Peripheral blood mononuclear cells and macrophages have drawn much attention as novel cellular vehicles for gene therapies in which these cells are genetically modified *ex vivo* and then reintroduced into the body (6). Furthermore, cationic liposome/DNA complexes have been shown to be capable of transfecting monocytes/macrophages *in vivo* in blood, liver, and spleen (7). These observations suggest that after systemic intravenous injection of a cationic liposome/DNA complex, circulating monocytes/macrophages could take up the introduced gene and infiltrate infarcted tissue. Therefore we tried to develop the systemic gene therapy using infiltrating macrophages as cell vehicles. We used an enhanced green fluorescent protein (EGFP) expression vector complexed with cationic liposomes for systemic gene therapy. After systemic administration of pIRES-EGFP plasmid vector with Lipofectin into normal rats, no EGFP-positive cells or macrophages were observed in intact brain. However, macrophages markedly accumulated in the brain tissue once infarct developed (Fig. 1C), and large numbers of EGFP-positive cells were detected in the marginal zone of the infarct. Expression of the exogenous EGFP gene was

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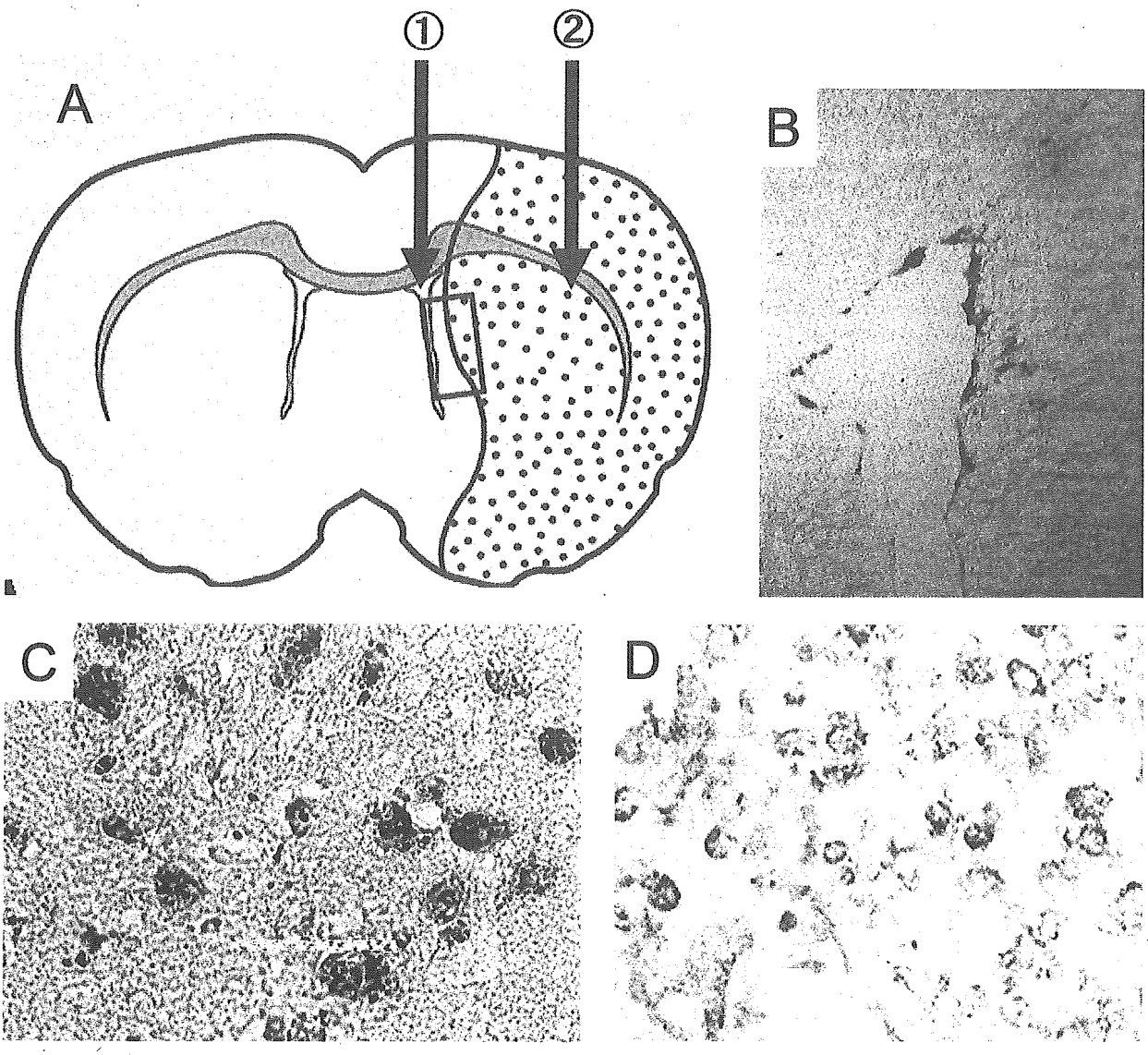

Figure 1. The diagram depicts sections of the rat brain after middle cerebral artery (MCA) occlusion with the infarct shown as stippled (A). The border area is indicated by the rectangular box in the striatum. Intraventricular (1) or intracerebral (2) injection is widely used for gene transfer into the brain. Intraventricular administration of adenoviral reporter gene resulted in expression of exogenous gene on the wall of the lateral ventricule (B). Macrophages accumulating along the margin of the evolving infarct are shown with anti-Mac2 antibody in (C). Immunohistochemistry with anti-EGFP antibody was used to confirm EGFP protein expression in the ischemic caudoputamen after intravenous injection of pIRES-EGFP plasmid vector (D).

confirmed immunohistochemically using an anti-EGFP antibody (Fig. 1D). Most EGFP-positive cells expressed monocyte/macrophage specific antigens. To deliver exogenous FGF-2 gene to the infarct, we injected pIRES-FGF2-EGFP plasmid. Marked expression of both FGF-2 and EGFP was observed in the infarct (Fig. 2A–C). Administration of pIRES-FGF2-EGFP plasmid increased the number of neural progenitor cells (Fig. 2D, 2E) in the lateral wall of the SVZ after MCA occlusion (Fig. 2F).

Gene therapy for stroke holds promise because of its ability to induce expression of desired molecules by cells for a long period. Gene transfer for neurotrophic factors (8), antiapoptotic protein (9), and heat shock protein (10) can ameliorate ischemic brain damage when administered before or even after induction of ischemia. Post ischemic treatment could be given to stroke patients provided that efficacy and safety were proven. However, the viral vectors such as herpes simplex virus and adenovirus used in experimental stud-

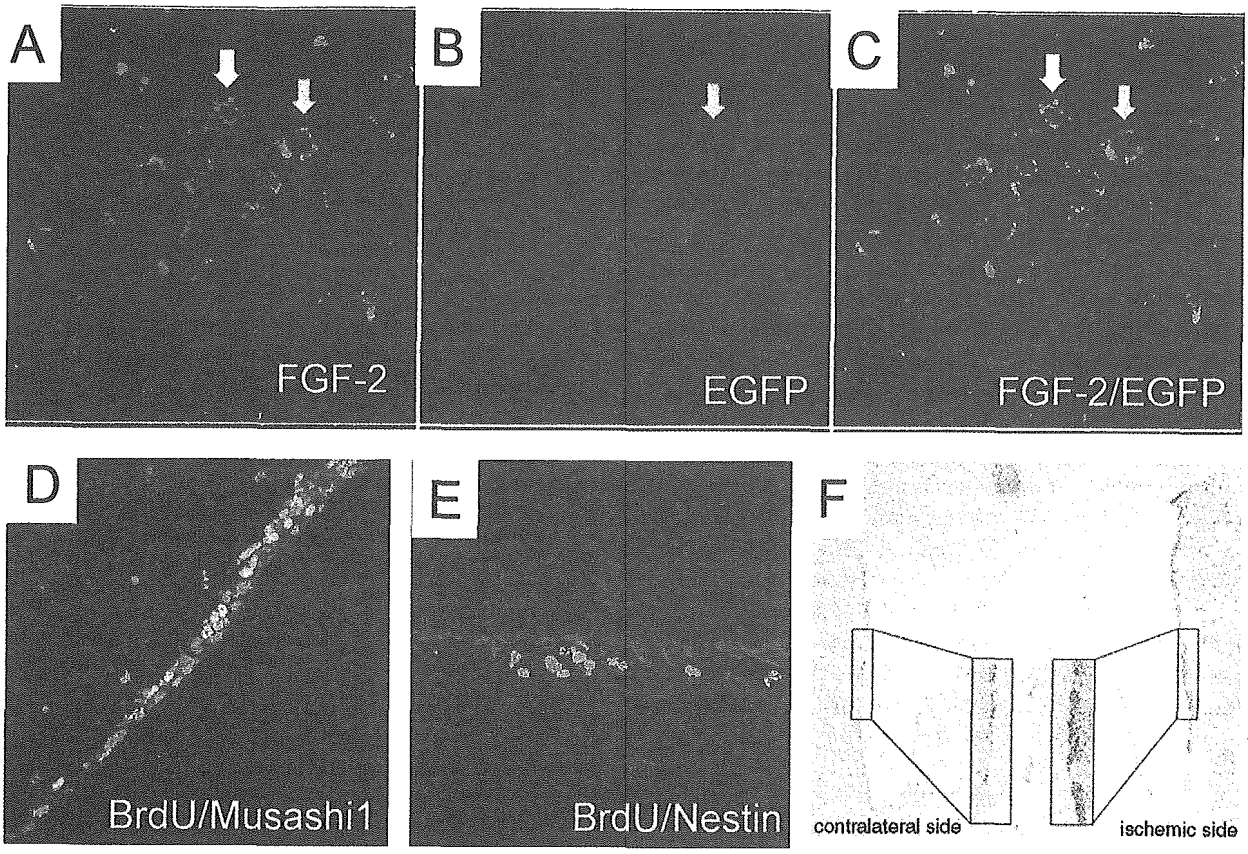

Figure 2. Systemic administration of the pIRES-FGF2-EGFP plasmid resulted in expression of FGF-2 and EGFP in experimental cerebral infarction. After injection with pIRES-FGF2-EGFP, most EGFP-positive cells also expressed FGF-2 (A to C; yellow arrow). However, endogenous FGF-2 expression also was seen as FGF-2-positive and EGFP-negative cells (white arrow in A and C). (D, E) Neural stem/progenitor cells in the SVZ. Cells doubly positive for BrdU plus either marker of neural stem/progenitor cells, Musashi-1 (E) or nestin (F), were localized in the SVZ. (F) Effect of systemic administration of pIRES-FGF2-EGFP plasmid on BrdU incorporation in the SVZ *in vivo* is shown. The plasmid was injected after MCA occlusion. Note the marked increase in BrdU-positive cells in the SVZ on the ischemic side.

ies, carry important potential problems such as toxic inflammatory responses, immunogenicity of virally infected cells, neoplastic transformation, relatively low DNA size limits, and difficulties in preparation. Furthermore, previous studies often have involved intracerebral, intrathecal, or intraventricular injection of the exogenous gene; such invasive gene delivery techniques are not practical for routine medical treatment in stroke patients. To date, neither viral nor nonviral methods have been effective in transfecting intact brain after intravenous delivery. The main advantages of nonviral methods including liposome-DNA complexes are safety, ease of preparation, and ability to deliver DNAs of unlimited size. Zhu et al (1993) (11), Liu et al (1997) (7), and Templeton et al (1997) (12) all reported that injection of plasmid DNA in cationic liposomes into the tail vein of mice could induce efficient gene transfer in several organs including lung, spleen, heart, kidney, and liver. Vascular endothelial cells, monocytes, and macrophages are the cell types most commonly transfected by intravenous injection of cationic liposome-DNA complexes. However, uptake by the endothelial cells was nearly absent in the brain. Although few macrophages are found in the intact brain, the circulating monocytes and other mononuclear blood cells accumulate in the brain and differentiate into microglia and macrophages once infarction develops (5). As novel cellular vehicles for gene therapy, the macrophages previously have been genetically modified *ex vivo* and reintroduced into the body with the hope that some of them will migrate selectively to the site of disease (13). When we used FGF-2 plasmid for systemic gene therapy, treatment increased numbers of neural progenitors in the SVZ on the ischemic side. It was previously shown that intracerebroventricular administration of FGF-2 protein or cDNA increased the numbers of the progenitor cells in the SVZ (4, 14). However, other neurotrophic factors such as epidermal growth factor may be more effective in enhancing neurogenesis after stroke.

The procedure we introduced will be attractive in stroke patients because it is essentially noninvasive. However, several technical problems such as nonspecific expression and low efficiency for the gene transfer need to be addressed in the future. Transcriptional targeting of the transgene using the macrophage-specific promoters or promoters of the genes up-regulated only within the cerebral infarct, may prove essential for avoiding unwanted side effects. It is well known that liposome-DNA complexes usually show low transfection efficiency *in vitro*. Modification of the cationic liposomes and the DNA to liposome ratio also might improve transfer efficiency. High-expression promoters could increase synthesis of the protein product, which might be otherwise insufficient when nonviral vectors are used for gene transfer.

In conclusion, we introduced intravenous delivery of the plasmid DNA to the macrophages infiltrating an experimental brain infarct in rats. Although further improvements in promoters for the expression vector, in liposomes complexed with the DNA, and in administration strategy will be required, systemic gene therapy using macrophages for *in vivo* targeting holds clinical promise in stroke patients, given its simplicity, safety related to the nonviral nature of the vector, and noninvasive mode of administration.

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Associations of Serum IL-18 Levels With Carotid Intima-Media Thickness

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Objective—Elevated circulating levels of IL-18 can predict future coronary heart disease. Although IL-18 is thought to play a crucial role in atherosclerosis, whether circulating IL-18 levels are associated with the severity of atherosclerosis remains to be determined. With the use of B-mode ultrasound, this study examines the relationships of serum IL-18 levels with carotid intima-media thickness (IMT) as a reflector for systemic atherosclerosis.

Methods and Results—The study comprised 366 patients without histories of cardiovascular accidents. Severity of carotid atherosclerosis was evaluated by the mean max IMT, ie, mean of the maximal wall thickness at 12 carotid segments. Serum IL-18, IL-6, and high-sensitive C-reactive protein (hs-CRP) levels were determined in all patients. Log-transformed IL-18 concentrations were positively correlated with IMT (r=0.36, P<0.001), and the association remained significant (β=0.20, P<0.001) when controlling for traditional atherosclerotic risk factors, IL-6 and hs-CRP levels. Also, IMT was greater in the highest and the middle tertile of IL-18 levels than in the lowest tertile.

Conclusion—Higher serum IL-18 levels appear to be associated with greater carotid IMT, suggesting the link between IL-18 and atherosclerosis. (***Arterioscler Thromb Vasc Biol.* 2005;25:1458-1462.**)

Key Words: atherosclerosis ■ carotid arteries ■ cytokines ■ inflammation ■ ultrasonic diagnosis

With the recognition that inflammation plays a significant role in atherosclerosis and its complications, studies have shown that circulating inflammatory markers are predictive of cardiovascular diseases (CVD).[1] Particularly, elevated circulating levels of C-reactive protein (CRP) and IL-6 (IL-6) have been associated with the risk for CVD.[2–4] Additionally, circulating levels of IL-18 are found to be a strong predictor of CVD deaths in patients with coronary artery disease,[5] as well as an independent predictor for coronary events in healthy men.[6]

IL-18 was originally identified as an interferon-γ–inducing factor,[7] which may play a central role in the inflammatory cascades.[8] To date, experimental studies have shown that expression of IL-18 is related with atherosclerotic plaque progression and its instability.[9,10] However, clinical studies relating IL-18 to atherosclerotic severity are limited.[11] Moreover, we are unaware of studies that examined the relationships with IL-6 and high-sensitive CRP (hs-CRP) taken into account.

Carotid intima-media thickness (IMT), as assessed by B-mode ultrasound, is a commonly used clinical marker that reflects systemic burden of atherosclerosis.[12] Moreover, increased IMT has been a predictor of future coronary events and stroke.[13–15] On the basis of such findings, to clarify the relationships between such inflammatory markers and IMT would be of value to extend the current knowledge regarding the link between inflammation and atherosclerotic diseases.

With the use of B-mode ultrasound, this study examines the relationships of serum IL-18 levels with carotid IMT as a reflector for systemic atherosclerosis.

Methods

Subjects

The subjects for this investigation were enrolled from patients of the Department of Internal Medicine and Therapeutics at Osaka University Hospital, who had undergone carotid ultrasound examination between October 2000 and November 2004.

In the current study, patients with the histories of ischemic heart disease, stroke or arteriosclerosis obliterans were excluded, because circulating IL-18 levels can be substantially modified in such patients.[16–18] Also, patients with acute inflammatory diseases, collagen diseases, or malignant neoplasm were excluded, because levels of inflammation can be greatly enhanced by such diseases. Additionally, patients with occluded carotid arteries and those with the history of carotid endarterectomy were excluded, because IMT could not be correctly determined in such patients.

Consequently, this study comprised 366 patients (mean±SD, age 64.8±9.1 years) equally including men and women. Patients' characteristics are shown in Table 1, demonstrating higher prevalence of atherosclerotic risk factors in the study sample. Institutional ethical committee approved this study, and written informed consent was obtained from all patients. Also, the investigation conforms to the principles outlined in the Declaration of Helsinki.

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TABLE 1. Patient Characteristics (n=366)

Age, y	64.8±9.1
Men, n (%)	180 (49)
Body mass index, kg/m^2	23.2±2.8
Hypertension, n (%)	262 (72)
Medical treatment, n (%)	195 (53)
ACEI or ARB use, n (%)	80 (22)
Systolic blood pressure, mm Hg	137±18
Diastolic blood pressure, mm Hg	80±12
Diabetes mellitus, n (%)	64 (18)
Medical treatment, n (%)	26 (7)
Fasting blood glucose, mmol/L	5.7±1.3
Dyslipidemia, n (%)	255 (70)
Medical treatment, n (%)	98 (27)
Statin use, n (%)	80 (22)
Total cholesterol, mmol/L	5.6±0.9
Triglyceride, mmol/L	1.5±0.8
HDL-cholesterol, mmol/L	1.5±0.4
Smokers, n (%)	169 (46)
Aspirin use, n (%)	45 (12)
Inflammatory markers	
IL-18, pg/mL (median)	194±81 (177)
IL-6, pg/mL (median)	1.96±2.38 (1.41)
hs-CRP, mg/dL (median)	0.13±0.23 (0.06)
Mean max-IMT, mm	0.99±0.27

Error terms are SD. ACEI indicates angiotensin-converting enzyme inhibitors; ARB, angiotensin II type1 receptor blocker.

Carotid Ultrasonography

All ultrasound examinations were performed with the use of Phillips SONOS 5500 equipped with a 3- to 11-MHz linear-array transducer. Three different longitudinal (anterior oblique, lateral, and posterior oblique) and transverse images of the bilateral carotid arteries were obtained, and IMT was measured as the distance between the luminal–intimal interface and the medial–adventitial interface. It was measured with the use of an electronic caliper on the frozen frame of a suitable longitudinal B-mode image in which putative maximal IMT was visualized. Thereby, severity of carotid atherosclerosis was evaluated by the mean max-IMT, which is the mean of maximal wall thickness at 12 carotid segments (near and far wall of the left and right common carotid artery, carotid bifurcation, and internal carotid artery).

All examinations were performed by one sonographer (H.Y.) who was blinded from the patients' clinical details. Before this study, reproducibility of the mean max-IMT was examined for randomly selected 70 patients without carotid occlusion, in which IMT measurements were performed twice by the same examiner. Intraclass correlation coefficient for the mean max-IMT measurements was 0.96, with a similar average between the two measurements.

Measurement of Serum Inflammatory Markers

After carotid ultrasound examinations, blood was drawn with minimally traumatic venipuncture for the measurement of serum inflammatory markers. Thereafter, the blood was centrifuged at 3000 rpm at 4°C for 15 minutes, and aliquots were stored at −70°C. Serum concentration of IL-18 was measured by single determination with enzyme-linked immunosorbent assay method (MBL Co, Ltd. Nagoya, Japan). In this assay system, mean interclass coefficient of variation (CV) of IL-18 measurements was 5.9%. Also, in 52 randomly selected patients, within-person correlation coefficient by 1-year interval was 0.84 ($P<0.001$).

Additionally, level of IL-6 was measured by enzyme-linked immunosorbent assay method (R & D system, Minneapolis, Minn), and hs-CRP was measured by latex turbidimetric immunoassay (Shionogi Biomedical Laboratory Inc, Osaka, Japan).

Evaluation of Atherosclerotic Risk Factors

Levels of fasting blood glucose, serum total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides (TG) were determined from the blood sample taken for inflammatory marker evaluations. Information on the patients' medical histories and medication usages was obtained from the clinical records. Hypertension was defined by casual blood pressure ≥140/90 mm Hg or the current use of antihypertensive agents. Diabetes mellitus was defined by fasting blood glucose ≥7.0 mmol/L or by the use of glucose-lowering agents. Dyslipidemia was defined by fasting serum total cholesterol >5.7 mmol/L, TG >1.7 mmol/L, HDL cholesterol <1.04 mmol/L, or by the use of cholesterol-lowering agents. Smoking status was categorically evaluated based on self-reports, with a smoker defined by the history of smoking ≥10 cigarettes per day >1 year.

Statistical Analyses

All analyses were performed with SPSS 11.5J (SPSS Japan Inc). Because distributions of inflammatory markers levels appeared to be left-skewed, they were normalized by log-transformation. Thereafter, associations between IL-18 levels and atherosclerotic risk factors were examined by Pearson correlation analysis for continuous variables, and by unpaired t test for categorical variables. Also, relationships between inflammatory marker levels and mean max-IMT were examined by Pearson correlation analysis. Subsequently, multiple linear regression analyses were used to examine associations between IL-18 levels and mean max-IMT: (1) by controlling for age and sex; (2) by additionally controlling for traditional atherosclerotic risk factors (body mass index, hypertension, diabetes, smoking status, total cholesterol, TGs, and HDL cholesterol); and (3) by further controlling for IL-6 and hs-CRP levels. Finally, mean max-IMT was compared across the IL-18 tertiles by the general linear model, followed by Bonferroni post-hoc test. Probability values were 2-tailed and were considered significant when <0.05.

Results

Associations between IL-18 levels and atherosclerotic risk factors are shown in Table 2. Levels of IL-18 were higher in men than in women, in patients with hypertension than in those without, and in patients with smoking history than in those without. Also, IL-18 was positively correlated with age, body mass index, and TGs, and negatively with HDL cholesterol. Additionally, IL-18 levels showed modest correlations with IL-6 and hs-CRP ($r=0.23$ and 0.29; both $P<0.001$). Of note, IL-18 levels were similar between patients on aspirin, statins, angiotensin-converting enzyme inhibitors, or angiotensin II type 1 receptor blocker, and those not using them (data not shown).

To clarify the link between IL-18 and severity of atherosclerosis, associations of serum IL-18 levels with the mean max-IMT were examined. By univariate analysis, log-transformed concentration of IL-18 was positively correlated with IMT ($r=0.36$, $P<0.001$). By multiple regression analyses (Table 3), the association between IL-18 and IMT remained significant when controlling for age and sex (model 1), and additionally controlling for traditional atherosclerotic risk factors (model 2). Moreover, the association was little attenuated when further controlling for IL-6 and hs-CRP (model 3). Of note, although both IL-6 and hs-CRP had

TABLE 2. Associations of Serum IL-18 Levels With Atherosclerotic Risk Factors (n=366)

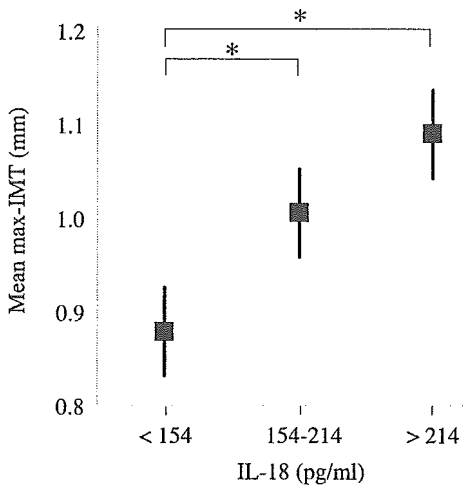
	r or Mean±SD	P
Age, y	0.17	0.001
Sex, men/women	208±85/180±74	<0.001
Body mass index, kg/m²	0.11	0.041
Hypertension, yes/no	202±82/173±73	<0.001
Systolic blood pressure	0.16	0.002
Diastolic blood pressure	0.01	0.81
Diabetes mellitus, yes/no	205±92/191±78	0.37
Fasting blood glucose	−0.01	0.88
Dyslipidemia, yes/no	195±81/191±81	0.58
Total cholesterol	−0.10	0.06
Triglyceride	0.10	0.056
HDL-cholesterol	−0.17	0.001
Smoking, yes/no	206±86/183±74	0.008
IL-6, pg/mL	0.23	<0.001
hs-CRP, mg/dL	0.29	<0.001

Levels of inflammatory markers were analyzed as log-transformed values. HDL indicates high-density lipoprotein.

significant correlations with carotid IMT (r=0.25 and r=0.23, both P<0.001), neither of such associations was significant when IL-18 and traditional atherosclerotic risk factors were simultaneously included in the model (model 3).

Additionally, given different IL-18 levels between men and women, we have performed separate analyses. Similar to the results obtained for all 366 patients, IL-18 levels were correlated with IMT both in men (r=0.36, P<0.001) and in women (r=0.31, P<0.001), and the associations remained significant when controlling for age, body mass index, and traditional atherosclerotic risk factors (in men, β=0.18, P=0.01; and in women, β=0.25, P<0.001).

Given the association between IL-18 and carotid IMT, the mean max-IMT was compared across the tertiles of IL-18 levels (Figure). IMT was greater in the highest and the middle



Mean max-IMT according to tertiles of IL-18. Error bars are 95% CI. *P<0.001

tertile of IL-18 than in the lowest tertile (Table 4). Moreover, the differences persisted when adjusting traditional atherosclerotic risk factors, log-transformed IL-6 and hs-CRP, and medication usages.

Discussion

In the present study, we have found that elevated serum IL-18 levels are associated with increased carotid IMT as evaluated by B-mode ultrasound. Also, the association was independent of traditional atherosclerotic risk factors, IL-6, and hs-CRP levels. To our knowledge, this is the first study that demonstrates the associations between IL-18 levels and carotid atherosclerosis, with IL-6 and hs-CRP taken into account.

In the current study, IL-18 levels were higher in hypertensive patients and in smokers than in those who were not, and had significant correlations with traditional atherosclerotic risk factors (Table 2). These findings are approximately in line with those of Ferrucci et al,[19] who showed associations of higher IL-18 levels with such risk factors. Also, in accor-

TABLE 3. Multivariate Analyses of Mean Max-IMT

	Model 1		Model 2		Model 3	
Variables	β	P	β	P	β	P
IL-18*, pg/mL	0.26	<0.001	0.22	<0.001	0.20	<0.001
Age, y	0.34	<0.001	0.36	<0.001	0.34	<0.001
Sex, men/women	0.21	<0.001	0.11	0.08	0.10	0.09
Body mass index, kg/m²			−0.08	0.10	−0.10	0.05
Hypertension, yes/no			0.17	<0.001	0.17	<0.001
Diabetes mellitus, yes/no			0.03	0.55	0.02	0.65
Total cholesterol, mg/dL			0.01	0.93	0.01	0.84
Triglycerides, mg/dL			0.01	0.83	0.01	0.84
HDL cholesterol, mg/dL			0.07	0.18	−0.05	0.33
Smoking, yes/no			0.14	0.021	0.13	0.026
IL-6,* pg/mL					0.05	0.34
hs-CRP,* mg/dL					0.08	0.16

*Levels of inflammatory markers were analyzed as log-transformed values.

TABLE 4. Mean Max-IMT Stratified by IL-18 Tertiles

	IL-18 tertile		
	Lowest (<154 pg/mL)	Middle (154–214 pg/mL)	Highest (>214 pg/mL)
Observed IMT, mm	0.88	1.00*	1.09*
95% CI	0.83–0.92	0.96–1.05	1.04–1.13
Adjusted IMT,‡ mm	0.92	1.01†	1.04*
95% CI	0.87–0.96	0.96–1.05	1.00–1.09
Adjusted IMT,§ mm	0.92	1.01†	1.04*
95% CI	0.88–0.97	0.97–1.05	1.00–1.08

*$P<0.005$ vs lowest tertile.

†$P<0.05$ vs lowest tertile.

‡When controlling for age, sex, body mass index, hypertension, diabetes mellitus, smoking status, total cholesterol, triglyceride and HDL cholesterol.

§When additionally controlling for use of stains, aspirin, and ACEI/ARB, and log transformed hs-CRP and IL-6.

dance with previous studies,[5,6,20] IL-18 levels had modest correlations with other inflammatory markers. Nevertheless, studies that examined the associations of IL-18 levels with atherosclerotic risk factors and other inflammatory markers are limited, requiring further studies to clarify their linkages.

Although elevated IL-18 levels can predict the development of CVD,[5,6] their association with carotid IMT remains to be examined. In the present study, we have found that higher IL-18 levels are associated with greater IMT, suggesting their link with carotid atherosclerosis. However, because of the impact of atherosclerotic risk factors,[21–23] the association between IL-18 and IMT may need to be examined with such factors taken into accounts. When controlling for age and sex, IL-18 was significantly associated with IMT (Table 3, model 1), and the association was independent of traditional atherosclerotic risk factors (Table 3, model 2). Furthermore, similar results were obtained when separate analyses were performed for men and women. These findings support the association between IL-18 and carotid atherosclerosis. Additionally, such association was little modified when levels of IL-6 and hs-CRP were further controlled for (Table 3, model 3), suggesting that the association is independent of such inflammatory markers. Of note, despite the associations of IL-6 or hs-CRP with IMT,[24–26] neither had significant association with IMT in the multiple regression model, which could be because of the lack of our statistical power.

To further demonstrate the associations between carotid atherosclerosis and IL-18, mean max-IMT was compared across the tertiles of IL-18 levels. IMT was greater in patients belonging to the highest and the middle tertiles than in those belonging to the lowest tertile (Figure, Table 4), and the differences persisted when adjusting traditional atherosclerotic risk factors. The greater IMT in patients with higher IL-18 appears to be congruent with Aso et al[11] Moreover, although inflammatory markers levels can be modified by aspirin, statins, angiotensin-converting enzyme inhibitors, or angiotensin II type 1 receptor blocker,[27–30] the differences between IL-18 and IMT were not virtually modified when such medication usages were considered (Table 4), further supporting the link between IL-18 and carotid IMT.

IL-18 is highly expressed in human carotid atherosclerotic plaques, predominantly colocalized with macrophages.[9] Thus, increased IL-18 production from severe atherosclerotic lesions could contribute to the higher IL-18 found in this study. Also, experimental studies have shown that IL-18 enhances atherosclerosis through release of interferon-γ[10] and induces expression of IL-6 in vascular endothelial and smooth muscle cells.[31] Inversely, IL-18 deficiency reduces the extent of atherosclerosis in apolipoprotein E–knockout mice.[32] These findings are in accordance with the hypothesis that IL-18 plays a key role in atherogenesis, supporting the link between IL-18 and carotid atherosclerosis.

There are some limitations for the current study. First, because this study is cross-sectionally designed, we cannot determine the causal relationships between higher IL-18 levels and greater IMT. Second, we used single blood sampling for the measurements of IL-18 levels, which does not guarantee the average levels in our patients. However, our IL-18 measurements were relatively stable over 1 year, supporting the link between IL-18 and chronic atherosclerosis. Third, this study included substantial number of patients on medications, requiring larger studies to separate the effects of such medications.

In conclusion, we have demonstrated an association between higher serum IL-18 level and greater carotid IMT, suggesting the link between IL-18 and atherosclerosis. This finding can offer a clue to understand the role of IL-18 in the development of atherosclerotic diseases.

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Critical Analysis of Hemodynamic Insufficiency by Head-up Tilt in Patients With Carotid Occlusive Disease

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Background The objective of this study was to evaluate the diagnostic value of the head-up-tilt (HUT) test for detecting cerebral hemodynamic insufficiency in patients with major cerebral artery occlusion disease because such patients may benefit from extracranial–intracranial bypass surgery.
Methods and Results In 13 cases of transient ischemic attacks in patients with carotid or middle cerebral artery occlusive disease, the HUT test was used to determine whether or not the symptoms appear during induced hypotension before investigating cerebral hemodynamics with positron emission tomography. Three of the 13 patients showed focal symptoms such as hemiparesis and limb shaking during the HUT test. In all 3 patients, the oxygen extraction fraction (OEF) increased beyond 53.3% (ie, misery perfusion), whereas only 2 of the other 10 patients without focal symptoms showed an increase in OEF during HUT.
Conclusions The HUT test was highly useful for screening patients with cerebral hemodynamic insufficiency in carotid occlusive disease. (*Circ J* 2005; **69:** 971–975)

Key Words: Cerebral hemodynamics; Head-up-tilt; Positron emission tomography; Transient ischemic attack

In patients with ischemic cerebrovascular disease (CVD), atherothrombotic occlusion or severe stenosis of the major cerebral arteries can cause chronic hypoperfusion in the border zone area[1,2] However, the cause of ischemic stroke associated with previously occluded major vessels such as the internal carotid artery (ICA) would be largely emboli either from the distal or proximal stump of the occluded vessel or from atherosclerotic plaque[3] For management of patients with transient ischemic attacks (TIA) or minor stroke with major vessel occlusion, it is critically important to clarify which mechanism, embolic or hemodynamic, underlies the pathophysiology. Cerebral hemodynamics can be assessed precisely with positron emission tomography (PET), and patients with misery perfusion[4] characterized by an increased oxygen extraction fraction (OEF), are at high risk for subsequent stroke[5,6] Although hemodynamic CVD is unlikely to occur in patients with normal hemodynamics, embolic TIA can occur in patients with hemodynamic cerebrovascular insufficiency. Therefore, careful history taking is also essential for diagnosing hemodynamic TIA; however, the number, stereotype, duration of neurological symptoms are unreliable indicators of impaired cerebral hemodynamics[7] Head-up tilt (HUT) tests have been often used to examine cerebral autoregulation in patients with vasovagal syncope[8] Because focal neurological signs in hemodynamic TIA are often precipitated by standing up[9–11] orthostatic stress with HUT tests may reproduce the clinical symptoms in patients with cerebral hemodynamic insufficiency.

We have investigated the clinical symptoms with HUT test and cerebral hemodynamics with PET in carotid TIA patients with major cerebral artery occlusive disease for determining an operation indication of extracranial–intracranial (EC-IC) bypass surgery. The purpose of this study was to determine the diagnostic value of HUT tests in detecting hemodynamic insufficiency (ie, misery perfusion).

Methods

Enrollment in this study began in February 2000, and ended in December 2001. A total of 26 carotid TIA patients were hospitalized during this period at the Osaka University Hospital. Carotid TIA was diagnosed according to the National Institute of Neurological Disorders and Stroke classification of CVD III[12] Each subject underwent neurological and neuroradiological evaluations, including an evaluation for occlusive CVD by duplex carotid ultrasonography (US),[13] magnetic resonance imaging (MRI), magnetic resonance angiography (MRA), and cerebral angiography. The MRI examination was performed in 5-mm-thick sections along the orbitomeatal plane with a 1.5-T unit. Infarction was defined as a focal area with prolonged T1 and T2 relaxation times. After evaluation of major cerebral vessels with US, MRA or angiography, 14 patients (9 men, 5 women; mean±SD age, 57.9±12.3 years) with occlusion or stenosis of the ICA or the main trunk of the middle cerebral artery (MCA) were included in this study. The patients gave written informed consent to undergo

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