Inapparent Hemodynamic Insufficiency Exacerbates Ischemic Damage in a Rat Microembolic Stroke Model

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Background and Purpose—Patients with severe carotid artery stenosis may have more severe ischemic damage after embolic stroke than patients without this abnormality. Unilateral proximal carotid occlusion (UCO) alone typically does not induce infarction in normotensive rats. The aim of this study was to investigate whether UCO increases infarct size after microembolic, experimental stroke.

Methods—Microembolic infarction was induced in 2 groups of Sprague-Dawley rats by injecting 2000 microspheres (50-μm diameter) intracranially from the external carotid artery. The common carotid artery (CCA) was either ligated just after the injection (CCA occlusion group, n=8) or left intact (CCA open group, n=8). In the control group (n=4), vehicle without microspheres was injected and the CCA was ligated. Twenty-four hours later, the brains were removed and infarct volumes measured. Perfusion-weighted imaging was used to evaluate the cerebral circulation before and after CCA occlusion with and without microsphere injection in a separate group of animals (n=16).

Results—All animals in the microemboli groups survived and had only a slight hemiparesis 24 hours after occlusion. No neurological deficits were observed in the control group. Infarct volumes were 145±57 mm³ in the CCA occlusion group and 45±26 mm³ in the CCA open group (P<0.01). There were no infarctions detected in the control group. Perfusion-weighted imaging showed that cerebral blood flow decreased after the CCA occlusion in both experiments with and without the microsphere injection.

Conclusions—UCO alone does not induce ischemic damage, but it worsens ischemic lesion size after multiple microemboli. This is probably due to the slight cerebral perfusion insufficiency caused by UCO. These results suggest that patients with cerebral hemodynamic insufficiency, such as those with severe carotid stenosis, may have increased ischemic damage after microembolic events. (Stroke. 2000;32:2494-2499.)

Key Words: cerebral infarction ■ hemodynamics ■ microspheres

Unilateral proximal carotid occlusion (UCO) alone typically does not induce any neurological deficits¹ or ischemic lesions in normotensive rats. Moreover, cerebral blood flow (CBF) does not decrease² or only slightly decreases after UCO.³,⁴ However, UCO plus an additional decrease of cerebral perfusion pressure does induce cerebral ischemic lesions.⁵ These observations imply that UCO in normotensive rats produces subclinical cerebral insufficiency.

The most common causes of cerebral atherothrombotic infarction are hemodynamic failure and embolic events.⁶-⁹ Microemboli are frequently detected in patients with carotid stenosis,¹⁰-¹² and these microemboli may be warning signs of stroke.¹³ Reports suggest that microemboli may be related to symptomatic cerebral infarction in stroke patients with carotid stenosis.¹⁴ Although cerebral infarction in patients with carotid stenosis is induced not only by hemodynamic events but also by microembolic events,⁸ it is unclear whether hemodynamic factors influence ischemic damage after embolic stroke. It is assumed that patients with severe carotid stenosis may have more severe ischemic damage after embolic events than do patients without these changes.

Microspheres can occlude small cerebral arteries in animal ischemic models, and a sufficient number of microspheres can induce cerebral infarction. Cerebral infarction models induced by the injection of microspheres are used for investigation of CBF,³ cerebral metabolism, edema,¹⁵-¹⁷ and neurological symptoms.¹⁸ It is possible to regulate the extent of ischemic injury by changing the number of microspheres.¹⁸ The etiology of this ischemic injury induced by microsphere injection is multiple microembolic events. This model may be useful for investigating microembolic ischemia. The aim of this study was to...
determine whether inapparent carotid compromise increases infarct size after microembolic events.

**Materials and Methods**

We used 36 male Sprague-Dawley rats weighing 300 to 350 g. All procedures were performed in accordance with institutional guidelines. Animals were anesthetized intraperitoneally with 400 mg/kg chloral hydrate. For all animals, PE-50 polyethylene tubing was inserted into the left femoral vein for injecting gadopentetate dimeglumine (Magnevist, Berlex Laboratories). Body temperature was continuously monitored with a rectal probe and maintained at 37.0°C with a thermostatically controlled heating lamp.

In the microsphere study, 20 rats were used to investigate ischemic damage after microsphere injection. The right common carotid artery (CCA), external carotid artery (ECA), and internal carotid artery (ICA) were exposed through a ventral midline incision in the neck. The right ECA was isolated, and the superior thyroid and occipital arteries were ligated with a 5-0 suture and transected. The distal portion of the ECA was ligated tightly with a 5-0 suture. The ICA was further dissected distally, and the right pterygopalatine artery was ligated with a 5-0 suture. A 0.2-mL suspension of polymer microspheres (diameter, 50.00 ± 0.65 μm; 10 000/mL of suspension, Bangs Laboratory) in 20% dextran solution was injected into the ICA through PE-10 polyethylene tubing previously inserted from the proximal portion of the ECA and was flushed with 0.2 mL of saline. During the injection, the CCA was temporarily occluded with a 3-0 suture around the CCA. After the injection the tubing was removed, and the CCA was then ligated.

Neurological evaluation was performed 24 hours after the induction of ischemia and scored on a 6-point scale, as described previously, which was modified from the scale proposed by Zea Longa et al: 0 = no neurological deficit, 1 = failure to extend forepaw completely, 2 = circling to the left, 3 = falling to the left, 4 = spontaneous walking with a depressed level of consciousness, and 5 = death.

Twenty-four hours after injection, the animals were anesthetized with an intraperitoneal injection of chloral hydrate (600 mg/kg of body weight) and decapitated. The brains were quickly removed and sectioned coronally into 8 slices, each with a 2-mm thickness. The brain slices were incubated for 30 minutes in a 2% solution of 2,3,4-triphenyltetrazolium chloride at 37°C and fixed by immersion in 10% buffered formalin solution. The 8 brain sections per animal were photographed with a coupled CCD camera (ECD-100HR computer camera, ELECTRIM Corp). The unstained area of the fixed brain sections was defined as infarcted. With the use of an image-analysis program (Bio Scan Optimas), the area of the infarcted region and of both hemispheres were calculated for each coronal slice. A corrected infarct volume value was calculated to compensate for the effect of brain edema. Corrected infarct area in a brain slice was calculated by subtracting the area of normal tissue in the ipsilateral hemisphere from the total area of the contralateral hemisphere. The corrected infarct volume was then calculated by multiplying the area by the slice thickness and summing the volumes from all slices. These measurements of infarct volume were done by 1 of the investigators without knowledge of group assignment.

The microsphere study consisted of a CCA occlusion group (n=8) and a CCA open group (n=8). In the cerebral perfusion study, 16 rats were used to investigate perfusion changes with and without microsphere injection before and after CCA occlusion by MRI. The experimental methods were almost the same as those of the microsphere study, but the proximal portion of the ECA was ligated in the group without microsphere injection. The same dose of microspheres was injected into the experimental group. A 3-0 suture was tied loosely around the right CCA. This suture was connected to a nylon line threaded through PE-280 tubing. The animal was fixed in a head-holding device equipped with an ear bar and tooth bar. PE-280 tubing was attached to the wall of the chest and abdomen. Then the rat and the holder were placed in the magnet. Anesthesia was maintained with 1.0% isoflurane delivered in air at 1.0 L/min. Temperature was monitored with a rectal probe and maintained at 37.0°C by thermostatically regulated air flow. CCA occlusion was achieved in the magnet by continuously withdrawing the nylon line, which tightened the suture around the CCA. After the MRI measurements, the CCA occlusion was confirmed visually outside the magnet. The MRI experiments were performed in a GE CSI-I 2.0-T/45-cm imaging spectrometer (GE NMR Instruments) operating at 85.56 MHz for 1H and equipped with ±20 G/cm self-shielding gradients. T2-weighted echo-planar imaging was used to perform dynamic contrast-enhanced, perfusion-weighted imaging. Four contiguous coronal slices, with a 2-mm thickness, were acquired with a field of view of 25.6×25.6 mm and a matrix size of 64×64 (repetition time=900 ms, echo time=74 ms, echo-planar imaging data acquisition time=65 ms, number of excitations=1).

A total of 40 images was obtained for each slice. A bolus injection of 0.25 mL of gadopentetate dimeglumine was administered after the 15th image acquisition. The perfusion-weighted imaging data were processed to obtain estimates of the relative cerebral blood volume (rCBV) and vascular transit time. Although measurement of absolute CBV requires knowledge of signal intensity changes in the brain, a relative measurement is sufficient to follow changes in CBV. The CBF index (CBFi) was derived from the values of the determined rCBV and vascular transit time, where CBFi=rCBV/vascular transit time.

Perfusion-weighted imaging data were acquired before and 60 minutes after CCA occlusion. A region of interest analysis was performed by using NIH Image software (National Institutes of Health, Bethesda, Md). The region of interest was chosen in 4 contiguous slices to include the whole hemisphere, both ipsilateral and contralateral to the CCA occlusion, to measure rCBV and CBFi values at each time point. CBFi and rCBV ratios were calculated by dividing the ipsilateral whole-hemisphere values by the contralateral values.

The cerebral perfusion study consisted of 2 experimental groups. In 1, microspheres were injected (2000, n=8) to investigate the cerebral vascular effect of combined microsphere injection and CCA occlusion. The other group was not subjected to microsphere injection (n=8) to examine the effect of CCA occlusion only on perfusion.

The values presented in this study are mean±SD. A statistical analysis was performed with an unpaired t test for the physiological variables and infarction volumes. The Mann-Whitney U test was used for comparison of neurological scores. A paired t test was used for analyzing the cerebral perfusion data. Results were considered significantly different at values of P<0.05.

**Results**

No significant differences were detected in rectal temperature, mean arterial blood pressure, arterial pH, PaCO2, and PaO2 at both baseline and 60 minutes after injection in the microsphere study (Table 1). All animals survived for 24 hours after injection. No neurological deficits were observed in the control animals. The neurological score at 24 hours after injection was 1.3±0.5 (median, 1.0; range, 1 to 2) in the CCA open group and 2.8±1.3 (median, 3.0; range, 1 to 4) in the CCA occlusion group. The score in the CCA occlusion group was significantly worse than that of the CCA open group (P<0.01).
By 24 hours after microsphere injection, triphenyltetrazolium chloride–stained brain slices demonstrated patchy, unstained areas of infarcted tissue in the region supplied by the ipsilateral middle cerebral artery and anterior cerebral artery. The unstained, infarcted area in the CCA occlusion group was larger than that in the CCA open group. The volume of infarction was $45.6^{\pm}26$ mm$^3$ in the CCA open group and $145.6^{\pm}57$ mm$^3$ in the CCA occlusion group ($P<0.01$, Figure 1). No infarction was seen in the control group (with CCA occlusion but no injection of microspheres).

In the cerebral perfusion study in the group that was not subject to microsphere injection, although the rCBV and CBFi maps did not demonstrate obvious changes before and after CCA occlusion, the CBFi ratio decreased ($1.00^{\pm}0.03$ before CCA occlusion and $0.96^{\pm}0.06$ after CCA occlusion; $P<0.05$ by paired $t$ test) and the rCBV ratio increased ($0.98^{\pm}0.03$ and $1.02^{\pm}0.03$, respectively; $P<0.05$ by paired $t$ test). In the group with microsphere injection, patchy areas of perfusion deficits were observed before CCA occlusion, and these deficits increased after CCA occlusion. The CBFi ratio decreased significantly after CCA occlusion ($0.85^{\pm}0.04$ before CCA occlusion and $0.79^{\pm}0.04$ after CCA occlusion; $P<0.01$ by paired $t$ test; Figure 2).

**Discussion**

There are 2 predominant mechanisms for cerebral atherothrombotic lesions to produce infarction. In the first, the plaque enlarges to severely compromise the lumen of the artery, leading to a reduction of CBF (hemodynamic compromise). In the second, the atherosclerotic plaque causes infarction by embolization of thrombus or plaque fragments. Embolic signals in cerebral arteries are detected by transcranial Doppler not only in patients with cardioembolic stroke but also in those with atherothrombotic infarction. Recent studies with this method have demonstrated that there are many “silent” microemboli in the cerebral arteries of patients with carotid stenosis. A report has suggested that the cause of symptomatic cerebral infarction in patients with carotid stenosis is a combination of cerebral hemodynamic failure and artery-to-artery embolism. Several studies have demonstrated that artery-to-artery embolization is the major cause of symptomatic cerebral infarction not only in those patients with high-grade stenosis but also in those with plaque ulceration. These studies have suggested that emboli from a carotid artery lesion are also very important when considering the management of patients with atherothrombotic infarction. Although emboli are likely to be an important factor leading to cerebral infarction in patients with carotid stenosis.
Carotid stenosis, it is not clear what the influence of cerebral hemodynamic factors is on embolic ischemic events. It is relatively unusual to observe an obvious unilateral CBF reduction in association with unilateral carotid stenosis or occlusion, but it is not rare to detect a decrease in CO₂ vasoreactivity or an increase in CBV in patients with carotid occlusion or stenosis. Patients with severe carotid stenosis may have more severe ischemic damage after embolic events than do patients without these changes after a similar embolic event. Our perfusion study demonstrated that CBF decreased and rCBV increased slightly but significantly after UCO. Although these changes are very small, the effects of UCO may be larger than the changes themselves. Our results demonstrate that inapparent hemodynamic compromise exacerbates ischemic damage associated with multiple microemboli.

Carotid artery stenosis is frequently observed in patients with ischemic heart disease. Neurological complications after coronary artery bypass surgery with cardiopulmonary bypass occur commonly, and 1 of these complications is stroke. Although low cerebral perfusion pressure during the operation can result in ischemic brain damage, many microembolic signals can be detected by transcranial Doppler not only during bypass surgery but also during percutaneous transluminal coronary angioplasty. Although almost all these microemboli are asymptomatic, they are a likely cause of complications after coronary artery bypass surgery in some patients. Our results indicate that microemboli may be more likely to be symptomatic in patients with severe carotid stenosis or carotid occlusion. Coronary artery bypass surgery without cardiopulmonary bypass can reduce the frequency of embolic signals during surgery, and this approach may protect those patients with severe carotid stenosis.

UCO alone typically does not induce any neurological deficit or ischemic lesions in normotensive rats. Our perfusion MRI study demonstrated that CBF decreased slightly but significantly after UCO. Li et al showed that there was no significant CBF laterality after CCA occlusion by perfusion MRI in Sprague-Dawley rats, and that UCO did not produce pathological ischemic damage after occlusion. Li et al showed that there was no significant difference between the occluded and nonoccluded hemispheres. These observations suggest that under normal oxygenation, the cerebral autoregulation system is effective and CBF does not decrease unless hypoxia is superimposed. Furthermore, the level of cerebral perfusion pressure after normoxic UCO is almost within the range of cerebral autoregulation. Our perfusion study demonstrated that the CBV ratio increased after UCO. This result indicates that the compensatory response is effective under conditions of reduced cerebral perfusion pressure. Several studies concerning the changes in CBF before and after UCO in rats have been conducted. Although some have demonstrated no significant difference in CBF between the hemispheres with and without CCA occlusion, de Ley et al reported that CBF decreases in both hemispheres were detected after CCA occlusion, but no CBF ratio change was observed before and after CCA occlusion. In our experiments, we compared the CBF right/left ratio but not absolute CBF values before and after UCO. Although the change in the CBF ratio might be underestimated because of CBF decreases in both hemispheres, our results demonstrate that ipsilateral CBF decreases after unilateral CCA ligation. Additional decreases in cerebral perfusion pressure, such as with hypotension or an increase in intracranial pressure, cause cerebral ischemia because perfusion pressure falls below the lower limit of autoregulatory capacity.

Small microspheres can occlude small cerebral arteries but not major cerebral arteries. A sufficient size and number of microspheres must be used to produce a reproducible cerebral infarction. To induce such ischemic damage, microspheres 35 to 80 µm in diameter are used. We selected a microsphere diameter of 50 µm because injection of microspheres <15 µm in diameter produces infarctions of variable size, and microspheres >70 µm in diameter produce multifocal microinfarctions. In this experiment, the number of injected microspheres was 2000. A pilot study had shown that injection of 500 microspheres produced little or no lesion, whereas 3000 microspheres induced massive lethal lesions (data not shown). Previously reported mortality rates in rats were 10% to 80% after the injection of various numbers (900 to 5000) and sizes (35 to 80 µm in diameter) of microspheres. Although all animals survived for 24 hours after injection in our experiment, a neurological score of 4 (no spontaneous walking, with a depressed level of consciousness) occurred in 3 of 8 animals in the CCA occlusion group. This observation suggests a reasonable neurological outcome compared with previous reports. Our perfusion study with the microsphere injection demonstrated that deficits increased after CCA occlusion and that the hemispheric CBF ratio decreased significantly after CCA occlusion. These findings suggest that CBF deterioration is the cause of the increase in lesion size.

In summary, UCO alone does not induce ischemic damage, but it exacerbates the size of ischemic lesions in our microembolic stroke rat model. These results suggest that patients with cerebral hemodynamic insufficiency, such as those with severe carotid stenosis, may have greater ischemic damage after microembolic events.

References
Editorial Comment

Large-vessel atherothromboembolism has been observed to account for between 15% and 20% of ischemic strokes in clinical trials. Hence, the observations reported by Omae and colleagues in the accompanying article are of interest to clinicians. Using a model of embolic stroke in the rat, the authors demonstrated that animals subjected to unilateral carotid occlusion before introduction of a standard dose of microemboli had larger infarct volumes than animals not subjected to occlusion. With MRI perfusion techniques, the authors demonstrated a minor, but statistically significant, asymmetry in hemispheric perfusion distal to the carotid occlusion, which they imply is the underlying reason for the difference in infarct volumes ultimately observed after embolization. While quantitative cerebral blood flow data might have been ideal, the authors’ observations argue for consideration of intervention in hemodynamically severe carotid stenosis in patients who might be subjected to an embolic stress, such as those undergoing coronary bypass surgery.

On the other hand, these data are potentially at odds with other reports of results of ischemic stress on brain tissue. Several experimental models, as well as some recent clinical observations, have suggested that prior ischemic stress may, in fact, be neuroprotective. While the mechanisms of this effect are poorly understood, upregulation of several genetically mediated cellular protective mechanisms has been suggested. In this regard, it would be of interest to see whether the authors’ model was also capable of demonstrating this effect, perhaps using sublethal doses of microemboli as the priming stimulus. If so, there must be a fine line between hemodynamic stress, which places tissue at risk, and transient ischemic stress, which is neuroprotective.

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