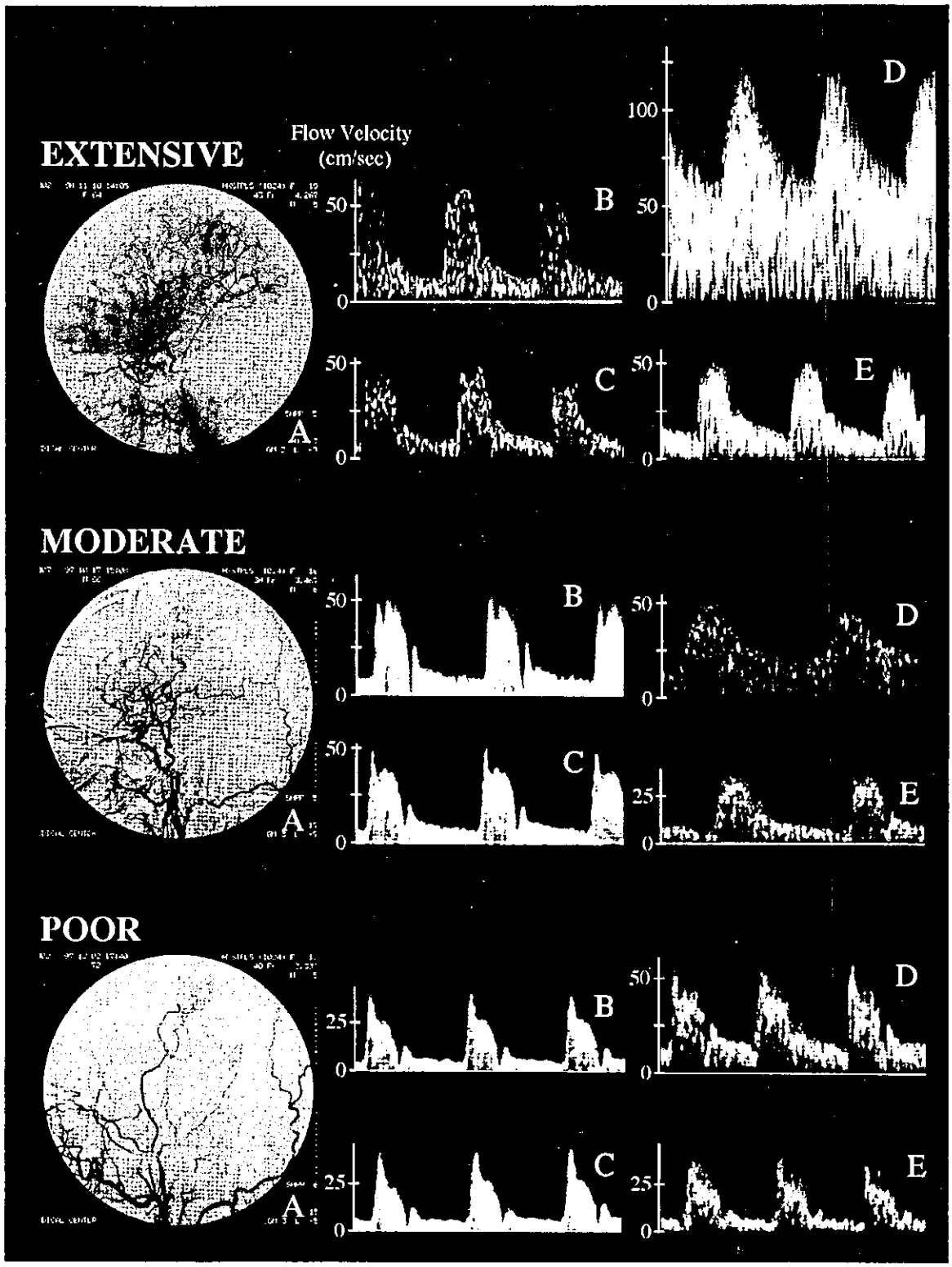

FIG 1. Postoperative digital subtraction angiograms and changes in pulsed Doppler ultrasonographic images of STA.

A, Postoperative digital subtraction angiograms. Pattern of collateral circulation through bypass was graded based on the findings of a postoperative angiogram of the external carotid artery.

B, Preoperative images of affected side.

C, Preoperative images of nonaffected side.

D, Postoperative images of affected side.

E, Postoperative images of nonaffected side.

Doppler wave forms of STA in a patient with extensive (*top*), moderate (*middle*), and poor (*bottom*) bypass flow are also shown. The vertical axis represents the flow velocity (in cm/s). Note that the postoperative EDV of the affected STA increased in patients with more extensive bypass flow. The postoperative EDV ratios of the anastomosed STA to the contralateral one was 3.9 (*top*), 2.1 (*middle*), and 1.5 (*bottom*), respectively.

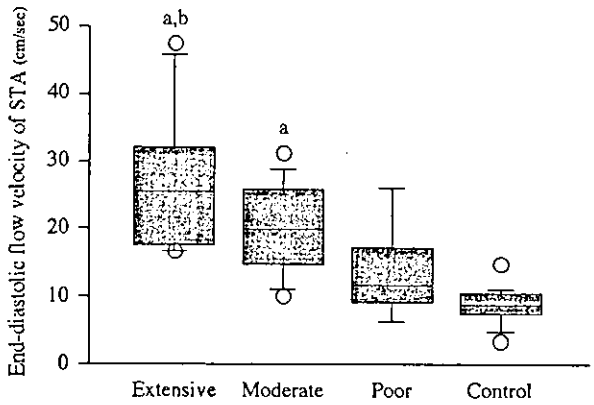

FIG 2. EDV of anastomosed STA after STA-MCA bypass surgery. Patients were divided into three subgroups according to the extent of bypass flow based on angiograms of the external carotid artery. Values of EDV were compared among groups. EDV was significantly higher in patients with more extensive bypass flow. *a*, $P < .0001$ versus control group; *b*, $P < .01$ versus poor group.

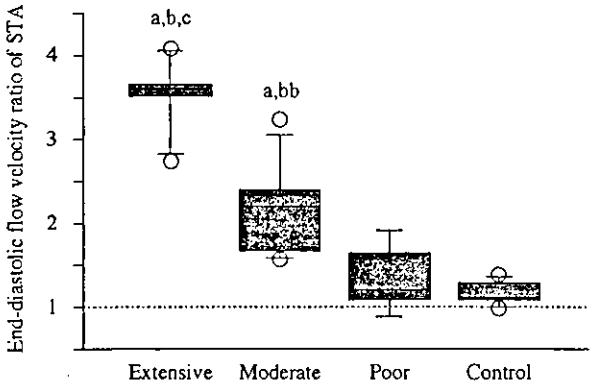

FIG 3. Ratios of EDV of affected STA to contralateral STA after STA-MCA bypass surgery in each group. Ratio of EDV was significantly higher in patients with more extensive bypass flow, and significant differences were observed between each group, except between the poor and control groups. *a*, $P < .0001$ versus control group; *b*, $P < .0001$ versus poor group. *c*, $P < .0001$ versus moderate group; *bb*, $P < .05$ versus poor group.

group (10 sides), a moderate group (16 sides), and a poor group (five sides). No patient was found to have an occluded bypass. Figure 1A shows the typical angiographic findings for each group.

Doppler Flow Velocity of STA and ECA

Various parameters in the flow velocity of bilateral STA and ECA were measured in the patients and control participants. The flow velocities measured in each artery were then compared according to the grades of the collateral pattern.

STA. The values of the PSV, EDV, and TMV of the anastomosed STA were 67.0 ± 12.0, 27.4 ± 8.8, and 41.7 ± 9.6 cm/s in the extensive group (n = 10), 53.8 ± 14.2, 23.0 ± 7.8, and 34.3 ± 10.2 cm/s in the moderate group (n = 16), and 46.6 ± 21.3, 13.5 ± 7.5, and 24.7 ± 11.2 cm/s in the poor group (n = 5), respectively. Those in the control group (n = 15) were 44.7 ± 8.7, 8.8 ± 2.8, and 18.9 ± 5.2 cm/s, respectively. The values of the PSV, EDV, and TMV of the anastomosed STA were significantly different among the groups ($P < .01$ for PSV, $P < .0001$ for both EDV and TMV by analysis of variance). The values tended to be higher in patients with more extensive bypass flow. This tendency was most evident in EDV (Fig 2). The values of the PI and RI of the anastomosed STA were 0.98 ± 0.26 and 0.59 ± 0.09 in the extensive group (n = 10), 0.93 ± 0.29 and 0.57 ± 0.09 in the moderate group (n = 16), and 1.33 ± 0.35 and 0.70 ± 0.09 in the poor group (n = 5), respectively. The values in the control group (n = 15) were 2.02 ± 0.64 and 0.81 ± 0.05, respectively. The values of the PI and RI of the anastomosed STA were significantly different among the groups ($P < .0001$ for both PI and RI by analysis of variance). The values of PI were significantly lower in the three patient groups than in the control group ($P < .0001$ for the extensive/moderate group versus control, $P < .05$ for the poor group versus control by Scheffe's multiple comparison test). The values of RI were significantly lower in the extensive and moderate groups than in the control group (both $P < .0001$ by Scheffe's multiple comparison test).

ECA. The values of PSV, EDV, and TMV of the affected ECA were 96.4 ± 32.1, 19.5 ± 10.6, and 41.3 ± 15.1 cm/s in the extensive group (n = 9), 91.0 ± 46.6, 17.6 ± 6.0, and 36.8 ± 14.7 cm/s in the moderate group (n = 16), and 96.4 ± 32.5, 15.0 ± 3.5, and 33.3 ± 6.3 cm/s in the poor group (n = 5), respectively. In one patient from the extensive group, the ECA flow velocity could not be measured because of the high position of the carotid bifurcation. The values in the control group (n = 15) were 73.8 ± 15.7, 10.0 ± 3.7, and 25.9 ± 7.1 cm/s, respectively. Concerning the affected ECA flow velocity, not PSV but EDV and TMV were significantly different among the groups ($P < .01$ for EDV and $P < .05$ for TMV by analysis of variance), thus indicating that the EDV and TMV of the affected ECA in the extensive and moderate groups were higher than those in the control group. However, no significant differences were observed among the patient groups. The values of the PI and RI of the affected ECA were significantly different among the groups ($P < .05$ for both PI and RI by analysis of variance, data not shown). Those in the extensive and moderate groups tended to be lower than those in the control group, although the differences were not significant.

Flow Velocity Ratio of STA and ECA

We next calculated the laterality ratio of the parameters of STA and ECA for the patients who underwent the bypass surgery unilaterally and compared them in each group.

STA. The PSV, EDV, and TMV ratios of STA were 1.7 ± 0.3, 3.4 ± 0.8, and 2.4 ± 0.5 in the extensive group (n = 8), 1.0 ± 0.3, 2.1 ± 0.5, and 1.5 ± 0.4 in the moderate group (n = 13), and 0.9 ± 0.3, 1.3 ± 0.4, and 1.2 ± 0.4 in the poor group (n = 5), respectively. Those in the control group (n = 15) were 1.0 ± 0.2,

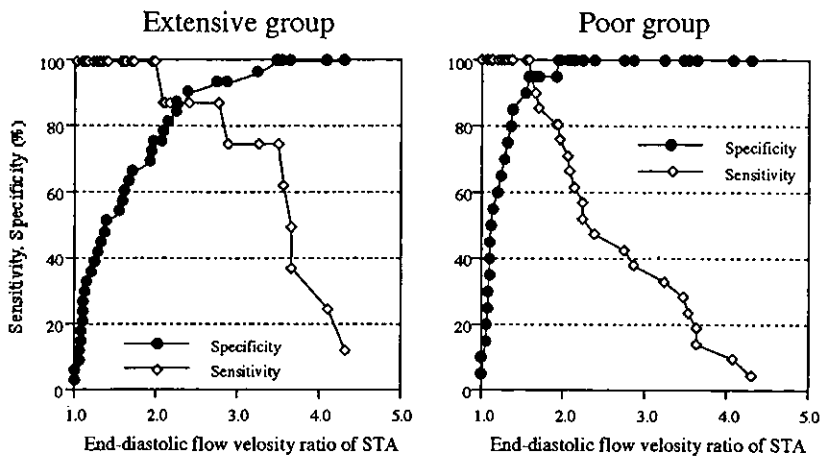

FIG 4. Sensitivity-specificity curve based on EDV ratio of affected STA to contralateral STA. Optimal threshold value of ratio for extensive group was 2.75 (*left*); that for poor group was 1.60 (*right*).

1.2 ± 0.1, and 1.0 ± 0.1, respectively. The PSV, EDV, and TMV ratios of STA were significantly different among the groups (all $P < .0001$ by analysis of variance), and they also tended to be higher in patients with a more extensive bypass flow. This tendency was most evident regarding the EDV ratio (Fig 3). The PI ratio was significantly lower in the three patient groups than in the control group ($P < .0001$ for extensive/moderate group versus control group, $P < .01$ for poor group versus control group by Scheffe's multiple comparison test, data not shown).

The RI ratio was significantly lower in the extensive and moderate groups than in the poor and control groups ($P < .0001$ for extensive/moderate group versus control group, $P < .05$ for extensive/moderate group versus poor group by Scheffe's multiple comparison test, data not shown).

ECA. Concerning the ECA, the flow velocity ratios were not different among the groups ($P = .6$ for PSV ratio and $P = .1$ for both EDV and TMV ratios by analysis of variance). On the other hand, the PI and RI ratios were significantly different among the groups ($P < .01$ for both PI and RI ratios by analysis of variance) and were lower in the extensive and moderate groups than in the control group ($P < .05$ for both the PI and RI ratios by Scheffe's multiple comparison test, data not shown).

Predictive Value for Detecting the Extent of Postoperative Bypass Flow

We analyzed the sensitivity-specificity curve for the relationship between the flow velocity ratio of EDV in STA and the grades of collateral circulation through the bypass. With this analysis, the optimal threshold value of this ratio between the extensive group and moderate group could be obtained as 2.75 and that between the moderate group and poor group as 1.60 (Fig 4). When the obtained values were applied, the sensitivity and specificity were 87.5% and 93.9% for the extensive group and 95.2% and 95.0% for the poor group, respectively.

Discussion

The extent of collateral flow through the anastomosed graft is not determined by the success of the procedure but rather by the preoperative hemodynamic state. Iwama et al (13) reported that the extent of bypass flow could be predicted from the preoperative hemodynamic status. They concluded that postoperative bypass function is expected only in patients with spontaneously developed leptomeningeal anastomoses and decreased reactivity to acetazolamide. In patients with lower perfusion pressure due to hemodynamically significant stenosis or occlusion, the stimulus for a good flow from an anastomosed graft tends to increase. On the other hand, those with preserved vasodilatory capacity have sufficient blood supply and, accordingly, a lesser demand for the additional flow via bypass. Yamashita et al (14) proposed that a reduced cerebrovascular reserve capacity or a reduced cerebral blood flow with a reduced cerebrovascular reserve capacity are the basic criteria for selecting candidates for bypass surgery. At present, a randomized controlled trial for EC/IC bypass is in progress to elucidate whether bypass surgery can effectively prevent ischemic stroke and impairment in the cognitive function in selected patients with decreased vasodilatory reserve (11, 12). Although the present study was performed based on this trial, its purpose was not to make any definitive conclusion but to provide new insight into the noninvasive assessment of bypass flow after surgery.

The effectiveness of the procedure can be directly judged by the luxuriance of flow in the MCA, visualized by using angiography. To evaluate the patency and extent of bypass flow after STA-MCA anastomosis, cerebral angiography is the standard for such assessment. Although cerebral angiography provides detailed information, it is invasive, time consuming, and requires the use of ionizing radiation. Because of these limitations, a noninvasive, real time, and easily repeatable technique that provides information regarding flow dynamics through the graft is preferable as an adjunct to the extracranial-intracranial bypass procedure. MR angiography has been reported to be an alternative for the assessment of bypass patency

(15). However, the information obtained by MR angiography is still limited and the procedure is expensive and not available at bedside. Instead, the use of duplex ultrasonography might offer a new tool for evaluating the hemodynamic state after STA-MCA anastomosis. The intraoperative measurement of blood flow in the bypass, performed by placing the transducer directly on the graft vessel, was reported to be useful for the assessment of patency during STA-MCA bypass surgery (16). However, this method is valid only during surgery. No data concerning the postoperative assessment of STA-MCA anastomosis are yet available. In the present study, we measured the flow velocities of STA and showed the possibility that the extent of bypass flow could be predicted by duplex ultrasonography.

In this study, we showed that the flow velocity of the anastomosed STA, particularly that in the end diastole, increased in patients with more extensive collateral flow through the STA-MCA bypass. The increase in the flow velocity of the STA may have reflected the increase in the cerebral blood flow through the bypass. The PI and RI of operated STA and ECA were significantly lower in the patients with more extensive collateral flow via graft bypass. Decreased PI and RI indicated that the blood flow pattern in anastomosed STA and ECA changed from a high resistance pattern to a low resistance one. Moreover, the present study clearly indicated that the EDV ratio of the operated STA to the contralateral one was a highly sensitive parameter for evaluating the extent of bypass flow in patients who underwent this procedure unilaterally. EDV ratios of STA >2.75 and <1.60 were found to indicate extensive and poor bypass flow, respectively. When these values are used, the sensitivity for detecting extensive and poor bypass flow was 88% and 95%, respectively. The specificity for both groups was 94% and 95%, respectively. The absolute value of the STA flow velocity, unlike the STA flow velocity ratio, was not significantly different between the moderate group and the two other patient groups. As a result, it was therefore difficult to estimate the extent of bypass flow based on the absolute value of the STA flow velocity. This may be because of the influence of systemic factors, including blood pressure and severity of atherosclerosis. The flow velocity of CCA also varied because of a conflict between the cerebral blood flow decrease due to carotid artery occlusive disease and the cerebral blood flow increase due to the STA-MCA bypass, and no relationship was observed with the extent of bypass flow (data not shown). Although the flow velocity of the ECA tended to be higher in the patient groups than in the control group, it did not show any significant correlation with the extent of bypass flow. We therefore could not predict the extent of bypass flow based on the ECA flow velocity. Moreover, measurement of the ECA flow velocity is sometimes difficult in patients whose carotid bifurcation is located in a high position. On the other hand, the STA flow velocity ratio clearly indicated the extent of bypass flow, because this ratio might correct for the influence of systemic factors.

Conclusion

We conclude that duplex ultrasonography is a potentially useful method for predicting the extent of collateral flow through an STA-MCA bypass. EDV ratios of STA >2.75 and <1.60 indicate extensive and poor bypass flow, respectively.

Acknowledgments

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Nicotinamide Attenuates Focal Ischemic Brain Injury in Rats: With Special Reference to Changes in Nicotinamide and NAD^+ Levels in Ischemic Core and Penumbra

Fumiko Sadanaga-Akiyoshi,[1,2,4] Hiroshi Yao,[1,2] Sei-ichi Tanuma,[3] Tatsuo Nakahara,[2] Jong Soon Hong,[1] Setsuro Ibayashi,[1] Hideyuki Uchimura,[2] and Masatoshi Fujishima[1]

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We investigated the neuroprotective action of nicotinamide in focal ischemia. Male spontaneously hypertensive rats (5–7 months old) were subjected to photothrombotic occlusion of the right distal middle cerebral artery (MCA). Either nicotinamide (125 or 250 mg/kg) or vehicle was injected IV before MCA occlusion. Changes in the cerebral blood flow (CBF) were monitored using laser-Doppler flowmetry, and infarct volumes were determined with TTC staining 3 days after MCA occlusion. In another set of experiments, the brain nicotinamide and nicotinamide adenine dinucleotide (NAD^+) levels were analyzed by HPLC using the frozen samples dissected from the regions corresponding to the ischemic core and penumbra. In the 250-mg/kg nicotinamide group, the ischemic CBF was significantly increased compared to that the untreated group, and the infarct volumes were substantially attenuated (−36%). On the other hand, the ischemic CBF in the 125 mg/kg nicotinamide group was not significantly different from the untreated CBF, however, the infarct volumes were substantially attenuated (−38%). Cerebral ischemia per se did not affect the concentrations of nicotinamide and NAD^+ both in the penumbra and ischemic core. In the nicotinamide groups, the brain nicotinamide levels increased significantly in all areas examined, and brain NAD^+ levels increased in the penumbra but not in the ischemic core. Increased brain levels of nicotinamide are considered to be primarily important for neuroprotection against ischemia, and the protective action may be partly mediated through the increased NAD^+ in the penumbra.

KEY WORDS: Cerebral ischemia; stroke; cerebral blood flow; poly (ADP-ribose) polymerase; apoptosis.

INTRODUCTION

Nicotinamide is the precursor of nicotinamide adenine dinucleotide (NAD^+), and it also can act as a poly(ADP-ribose) polymerase (PARP) inhibitor. In the case of DNA strand breaks, PARP is activated to synthesize poly (ADP-ribose) polymers with NAD^+ as a substrate, and thereby appropriate repair enzymes are able to repair the damaged DNA. When such broken DNA strands are too extensive, the inappropriate activation of PARP is thought to deplete the intracellular pools of NAD^+. As a result, the process of NAD^+ resynthesis subsequently lowers cellular ATP pools as

[1] Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.
[2] Center for Emotional and Behavioral Disorders, Hizen National Hospital, Saga, Japan.
[3] Department of Biochemistry, Faculty of Pharmaceutical Sciences, Science University of Tokyo, Tokyo, Japan.
[4] Address reprint requests to: Fumiko Sadanaga-Akiyoshi, MD, Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Maidashi 3-1-1, Higashi-Ku, Fukuoka 812-8582, Japan. Tel: +81-92-642-5256; Fax: +81-92-642-5271; E-mail: kamefu@rb-3.so-net.ne.jp

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four molecules of ATP are consumed in one NAD^+ resynthesis, thus leading to an ATP rundown (i.e., secondary energy failure and subsequent necrotic death) (1). In fact, PARP inhibitors such as benzamide derivatives have been demonstrated to play an important role in the attenuation of brain damage in cerebral ischemia (2–5). If this scenario is true, nicotinamide, which would increase brain levels of NAD^+, or by acting as a PARP inhibitor, could protect brain against cerebral ischemia.

Several lines of evidence support the secondary energy failure hypothesis, nonetheless there are some fundamental questions concerning the role of PARP activation in cerebral ischemia. Paschen et al. (6) reported that the ATP levels, the total adenylate pool, and the adenylate energy charge dropped to approximately 20%, 50%, and 40% of the control, respectively, whereas the NAD^+ levels remained close to the control levels in focal ischemia. These results do not support the hypothesis that a secondary energy failure resulting from a depletion of NAD^+ plays an important role in focal ischemic injury. Lin et al. (7) demonsrated that treatment with nicotinamide at a range of 7.5–15.0 mmol/L significantly protected the cultured hippocampal neurons from NO toxicity. In their study, nicotinamide directly prevented the activation of caspase 1 and caspase 3-like activities, and PARP cleavage after NO exposure (i.e., prevention of apoptotic process).

Although recent investigations have demonstrated that nicotinamide attenuates the infarct volume after focal cerebral ischemia (8–10), cerebral blood flow (CBF) was not examined in these studies. Because high doses of nicotinamide increase regional CBF (11), we need to investigate whether vasodilatation contributes to the neuroprotective effects of nicotinamide in focal cerebral ischemia. The purpose of our study was (i) to examine the effects of nicotinamide on the CBF and infarct size, and (ii) to determine the changes in nicotinamide and the NAD^+ levels in the brain after the IV injection of nicotinamide in rats with focal cerebral ischemia.

EXPERIMENTAL PROCEDURE

All procedures performed on the animals in the present study were done in accordance with the Animal Care Guidelines of Kyushu University. Forty-four male spontaneously hypertensive rats (SHR) (5–7 months old, 315–425 g body weight), were used in this study. The rats were maintained at the Kyushu University Animal Center under specific pathogen-free conditions and were fed regular rat chow (CLEA rodent diet CE-2) and tap water.

Photothrombotic Distal MCA Occlusion. We used the photothrombotic distal MCA occlusion model as previously described (12–14). Briefly, the rats were anesthetized with halothane (3% for induction, 1.5% during the surgical preparation with a face mask, 0.75% after intubation, and 0.5% for maintenance) in a mixture of 70% nitrous oxide and 30% oxygen. The right femoral artery and vein were cannulated using PE50 tubing. The rats were endotracheally intubated with PE240 tubing. Pancuronium bromide (an initial dose of 0.3 mg followed by 0.1 mg every 30 min) was injected IV, and the rats were mechanically ventilated. The mean arterial blood pressure (MABP) was continuously monitored, and physiological variables were determined before and after distal middle cerebral artery (MCA) occlusion. The rectal and head temperatures were maintained at approximately 37.5°C and 36.5°C, respectively, by means of a warming lamp. The rats were mounted on a stereotaxic head holder in the prone position, and a 2-cm incision was made vertically midway between the right orbit and the right external auditory canal. The temporalis muscle was separated and retracted to expose the zygoma and squamosal bones. Under an operating microscope (OPMI 111 Carl Zeiss, Oberkochen, Germany), a burr hole measuring 3 mm in diameter was made with a high-speed drill 1 mm rostal to the anterior junction of the zygoma and squamosal bones, thus revealing the distal segment of the MCA above the rhinal fissure. A thin bone layer was preserved to prevent injury to the brain and then was carefully removed with the forceps. The dura was thereby left intact. A krypton laser (Innova 301, Coherent Inc, CA, USA) operating at 568 nm was used to irradiate the distal MCA at a power of 20 mW. The laser beam was focused with a 30-cm focal length cylindrical lens (CKX 300, Newport Corporation, Tokyo, Japan) and positioned with a mirror onto the distal MCA. The photosensitizing dye rose bengal (Wako Pure Chemical Industries Ltd., Osaka, Japan, 15 mg/ml in 0.9% saline) was administered IV at a body dose of 20 mg/kg over a 90 second period simultaneously with 4 min of laser irradiation. One hour after distal MCA occlusion, the head wound was closed, and the catheters were removed. The rats were carefully weaned from the respirator, and returned to their home cage after regaining the ability to breathe independently.

Measurement of Regional CBF. The regional CBF was determined by laser-Doppler flowmetry (ALF 21D, Advance Co. Ltd., Tokyo, Japan) 10, 20, and 30 min after the start of the nicotinamide infusion, and 10, 30, and 60 min after MCA occlusion. CBF was measured at 1.0 mm posterior and 4.0 mm lateral to the bregma. The laser Doppler probe was placed in the penumbra. Our previous experiments demonstrated that after 30 min of distal MCA occlusion, each CBF was 72 ± 18%, 50 ± 14%, and 35 ± 11% of the control values at 2, 3, and 4 mm from the midline (15). Therefore the point where we monitored CBF (at 1 mm posterior and 4 mm lateral to the bregma) was considered to be the penumbral zone. Because visible light interferes with laser Doppler flowmetry, the heating lamp was temporarily turned off during the CBF measurements. Changes in the CBF were expressed as a percentage of the average of three baseline values.

Experiment 1. The rats were randomly divided into three groups: (i) untreated (equivalent volume of saline as vehicle) (n = 9), (ii) 125 mg/kg nicotinamide (n = 8), (iii) 250 mg/kg nicotinamide (n = 8). Each infusion was given intravenously for 30 min before MCA occlusion. Because our preliminary experiments revealed that spontaneous respiration did not recover in two of the three rats treated with 500 mg/kg of nicotinamide, we decided to use this drug at dosages of 125 and 250 mg/kg.

Three days after distal MCA occlusion, the rats were decapitated under amobarbital anesthesia (100 mg/kg IP), and then the

brain was rapidly removed. The entire brain was cooled in ice-cold saline for 10 min and cut into 2-mm-thick coronal sections in a cutting block. The brain slices were then immersed in 2% 2,3,5-triphenyltetrazolium chloride (Wako Pure Chemical Industries Ltd., Osaka, Japan) at 37°C for 30 min in the dark. The posterior surface of each section was photographed, and the infarct areas, indicated by a lack of staining, were determined with NIH Image software (Version 1.56). The infarct volume of each rat was calculated according to the trapezoidal rule (16).

Experiment 2. Fifteen SHR were randomly assigned to three groups as mentioned above. Physiological variables and CBF were determined as described in experiment 1. The brain was frozen *in situ* (17) 1 h after MCA occlusion with liquid nitrogen and was cut to produce a standard coronal block in a cryostat: four samples were dissected from the block as shown in Fig. 1a. In this stroke model, the therapeutic efficacy is consistently seen in the parietal cortex indicated as C in Fig. 1a and an asterisk in Fig. 1b. We therefore defined this region as a "penumbral" zone that can be rescued by therapeutic intervention. Our recent experiments showed that evolving "apoptotic" DNA fragmentation was found only in this C zone at 6 h after MCA occlusion (15). Frozen samples were stored at −80°C until the levels of nicotinamide and NAD^+ were measured by HPLC.

Quantification of NAD^+ and Nicotinamide. Trichloroacetic acid (TCA), nicotinamide, and β-NAD were obtained from Sigma (St. Louis, MO, USA). Tissue samples were homogenized in 5% TCA and then were centrifuged at 15,000 rpm for 5 min at 4°C. The supernatant was then pooled to measure the levels of nicotinamide and NAD^+. The precipitates were dissolved in 1 M NaOH, and the protein content was analyzed according to Lowry et al. (18) with bovine serum albumin as a standard. Twenty microliters of supernatant were injected into the HPLC system using an autoinjector (model 231-401, Gilson Medical Electronics, Villiers le Bel, France). Nicotinamide and NAD^+ were eluted from the C18 reverse-phase column (Tosoh, ODS-80T, Tokyo, Japan), with a linear acetonitrile gradient in a 0.05 M potassium phosphate buffer, pH 7.4, at a constant flow rate of 1.0 ml/min. The gradient was created by mixing solvents A and B, which were delivered using the Gilson solvent delivery system, 305 and 306 pumps, and an 811B Dynamic Mixer. The composition of eluent A was 0.05 M potassium phosphate buffer (pH 7.4), and that of eluent B was 2% acetonitrile in 0.05 M potassium phosphate buffer (pH 7.4). The gradient was 50%–80% over 5 min, thus allowing a sample analysis time of 20 min. Under these conditions, the quantification was based on the absorbance reading at 254 nm. The retention time was 7.5 min and 9.5 min for nicotinamide and NAD^+, respectively.

Statistical Analysis. The values were expressed as the mean ± SD except for error bars in Figs. 2 and 4. Differences in the physiological variables and infarct volume were analyzed with the unpaired Student's t-test. The levels of significance were set at conservative *P* levels according to the number of multiple comparisons (i.e., Bonferroni's principle).

RESULTS

The physiological variables in the experimental groups are shown in Tables I and II. There were no significant differences in MABP, head temperature, rectal temperature, blood gases, hematocrit, and blood glucose among the groups before MCA occlusion in both experiments 1 and 2. These physiological variables were not significantly different after the infusion of nicotinamide or saline and 10, 30, and 60 min after MCA occlusion.

Regional CBF did not change after nicotinamide (125 mg/kg, 250 mg/kg) infusion (Fig. 2). After MCA occlusion, the degree of regional CBF reduction in the 250 mg/kg nicotinamide group was significantly attenuated compared with that in the untreated group

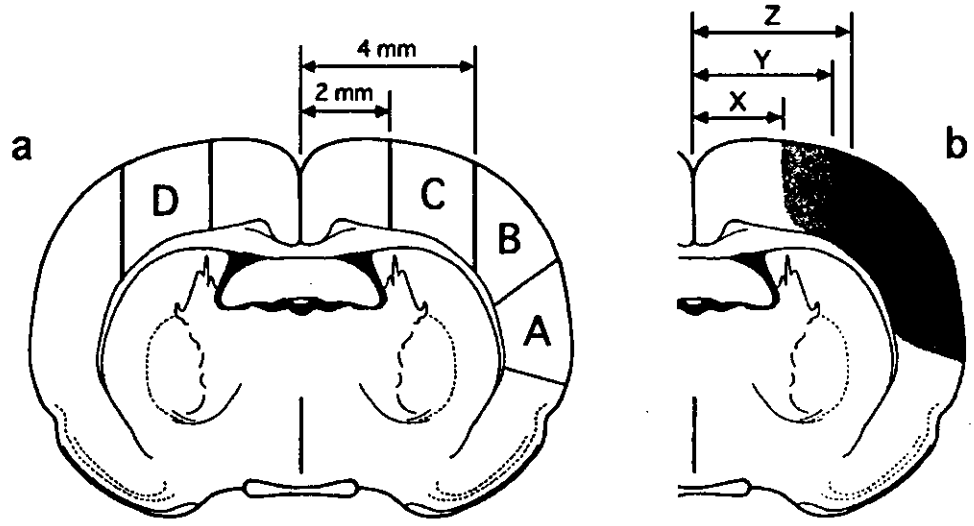

Fig. 1. a. Schematic drawing of a coronal slice of the brain. *A* and *B*, ischemic core: *C*, penumbra: *D*, non-ischemic zone. **b**, *X*, *Y*, *Z* indicate distance from the midline (mm) in untreated, nicotinamide 125-mg/kg, and 250mg/kg groups, respectively. $X = 2.01 \pm 0.47$, $Y = 2.91 \pm 0.62$, $Z = 3.11 \pm 0.74$. Values are mean ± SD. An asterisk shows penumbra area.

Table I. Physiological Variables in Experiment 1

	Resting	Drug*	MCA occlusion (min) 10	30	60
Untreated (n = 9)					
MABP (mm Hg)	178 ± 11	175 ± 9	209 ± 15	202 ± 11	188 ± 14
Head temperature (°C)	36.3 ± 0.1	36.3 ± 0.1	36.4 ± 0.1	36.3 ± 0.1	36.3 ± 0.1
Rectal temperature (°C)	37.4 ± 0.2	37.5 ± 0.1	37.5 ± 0.1	37.4 ± 0.1	37.4 ± 0.1
$PaCO_2$ (mm Hg)	39 ± 2	39 ± 4			42 ± 7
PaO_2 (mm Hg)	115 ± 14	118 ± 13			118 ± 14
pH	7.43 ± 0.03	7.42 ± 0.03			7.42 ± 0.05
Hct (%)	40 ± 2	40 ± 2			40 ± 2
Glucose (mM/L)	6.7 ± 0.9	6.9 ± 0.7			7.0 ± 1.2
Nicotinamide 125 mg/kg (n = 8)					
MABP (mm Hg)	180 ± 9	180 ± 10	203 ± 12	193 ± 14	184 ± 7
Head temperature (°C)	36.3 ± 0.1	36.2 ± 0.1	36.3 ± 0.1	36.3 ± 0.1	36.3 ± 0.1
Rectal temperature (°C)	37.4 ± 0.1	37.4 ± 0.1	37.3 ± 0.1	37.4 ± 0.1	37.4 ± 0.1
$PaCO_2$ (mm Hg)	40 ± 5	40 ± 4			40 ± 7
PaO_2 (mm Hg)	108 ± 13	106 ± 11			114 ± 10
pH	7.44 ± 0.05	7.45 ± 0.04			7.46 ± 0.05
Hct (%)	40 ± 2	40 ± 1			40 ± 1
Glucose (mM/L)	7.0 ± 0.9	6.5 ± 0.7			7.0 ± 1.7
Nicotinamide 250 mg/kg (n = 8)					
MABP (mm Hg)	180 ± 8	178 ± 10	206 ± 9	192 ± 10	186 ± 9
Head temperature (°C)	36.2 ± 0.1	36.2 ± 0.1	36.2 ± 0.1	36.2 ± 0.1	36.3 ± 0.1
Rectal temperature (°C)	37.3 ± 0.1	37.3 ± 0.1	37.3 ± 0.1	37.3 ± 0.1	37.3 ± 0.1
$PaCO_2$ (mm Hg)	40 ± 4	41 ± 6			40 ± 5
PaO_2 (mm Hg)	120 ± 20	117 ± 20			118 ± 14
pH	7.44 ± 0.03	7.44 ± 0.05			7.45 ± 0.05
Hct (%)	40 ± 1	40 ± 1			41 ± 1
Glucose (mM/L)	6.7 ± 1.4	6.9 ± 1.0			7.1 ± 1.9

Note: Values are the mean ± SD.
*30 min after nicotinamide or saline infusion.
MCA, middle cerebral artery; MABP, mean arterial blood pressure.

(37 ± 5% vs. 29 ± 5%, respectively, $P = .0090$). The ischemic CBF in the 125 mg/kg nicotinamide group was not significantly different from the untreated CBF.

The mean infarct volumes of the lower and higher doses of nicotinamide groups (52.0 ± 17.5 mm^3 and 53.6 ± 20.9 mm^3, respectively) were significantly smaller than those of the untreated group (84.2 ± 11.1 mm^3) (Fig. 3). Infarct attenuation by nicotinamide always occurred in the C zone, as shown in Fig. 1a, thus indicating that the penumbra exists in the C zone.

The levels of brain nicotinamide in the contralateral control regions were 1.36 ± 0.34 μ mol/g protein, and NAD^+ 1.14 ± 0.46 μ mol/g protein. Cerebral ischemia per se did not affect the concentrations of nicotinamide and NAD^+ in either the penumbra or ischemic core regions (Fig. 4). After the infusion of nicotinamide, the brain nicotinamide levels increased by 5.4- to 6.2-fold, and 8.7-to 12.4-fold in 125 mg/kg and 250 mg/kg groups, respectively, in comparison to those in the vehicle group (n = 5/group), in all areas (i.e., control area, penumbra, and ischemic core) (Fig. 4). In contrast, the brain NAD^+ levels increased in the penumbra (1.8- to 2.0-fold) and contralateral regions (2.0- to 2.2-fold), but not in the ischemic core (Fig. 4).

DISCUSSION

Pretreatment with the IV administration of nicotinamide reduced the infarct size in the model of focal cerebral ischemia. The infarct volumes determined 3 days after MCA occlusion were similarly attenuated both in the nicotinamide 125 mg/kg and 250 mg/kg groups in comparison to those in the untreated group. Although both the high and low doses of nicotinamide consistently reduced the degree of brain infarction at

Table II. Physiological Variables in Experiment 2

	Resting	Drug*	MCA occlusion (min) 10	30	60
Untreated (n = 5)					
MABP (mm Hg)	176 ± 7	177 ± 8	215 ± 11	195 ± 11	184 ± 8
Head temperature (°C)	36.4 ± 0.1	36.4 ± 0.1	36.3 ± 0.1	36.3 ± 0.1	36.3 ± 0.1
Rectal temperature (°C)	37.5 ± 0.2	37.4 ± 0.1	37.5 ± 0.1	37.5 ± 0.2	37.4 ± 0.1
$Paco_2$ (mm Hg)	38 ± 3	37 ± 1			36 ± 1
Pao_2 (mm Hg)	101 ± 8	108 ± 8			108 ± 11
pH	7.45 ± 0.01	7.46 ± 0.02			7.46 ± 0.01
Hct (%)	39 ± 3	39 ± 2			39 ± 2
Glucose (mM/L)	7.4 ± 0.7	7.0 ± 1.1			6.6 ± 0.4
Nicotinamide 125 mg/kg (n = 5)					
MABP (mm Hg)	175 ± 5	173 ± 4	210 ± 10	194 ± 7	184 ± 9
Head temperature (°C)	36.4 ± 0.1	36.4 ± 0.0	36.3 ± 0.0	36.3 ± 0.0	36.4 ± 0.1
Rectal temperature (°C)	37.5 ± 0.1	37.4 ± 0.1	37.4 ± 0.0	37.4 ± 0.1	37.5 ± 0.0
$Paco_2$ (mm Hg)	39 ± 2	36 ± 3			37 ± 1
Pao_2 (mm Hg)	100 ± 6	101 ± 9			104 ± 6
pH	7.44 ± 0.03	7.45 ± 0.03			7.47 ± 0.01
Hct (%)	40 ± 4	40 ± 3			40 ± 3
Glucose (mM/L)	7.7 ± 0.7	6.9 ± 1.1			6.2 ± 0.9
Nicotinamide 250 mg/kg (n = 5)					
MABP (mm Hg)	174 ± 7	170 ± 4	206 ± 11	183 ± 7	175 ± 6
Head temperature (°C)	36.4 ± 0.0	36.4 ± 0.1	36.3 ± 0.0	36.4 ± 0.1	36.4 ± 0.1
Rectal temperature (°C)	37.6 ± 0.1	37.5 ± 0.1	37.4 ± 0.0	37.4 ± 0.1	37.5 ± 0.0
$Paco_2$ (mm Hg)	37 ± 2	36 ± 1			36 ± 2
Pao_2 (mm Hg)	100 ± 7	104 ± 4			105 ± 9
pH	7.44 ± 0.02	7.46 ± 0.02			7.47 ± 0.02
Hct (%)	40 ± 3	39 ± 3			39 ± 3
Glucose (mM/L)	7.4 ± 0.3	6.3 ± 0.6			6.6 ± 1.1

Note: Values are the mean ± SD.
*30 min after nicotinamide or saline infusion.
MCA, middle cerebral artery; MABP, mean arterial blood pressure.

the penumbra, reduction of before CBF in the 250 mg/kg group was attenuated while the CBF in the 125 mg/kg group did not differ from the untreated CBF. These results indicate that the neuroprotective effect of nicotinamide against focal ischemia was already maximal at low doses where no significant vasodilative effect was observed.

The PARP hypothesis requires that a depletion of the NAD^+ pools precedes the depletion of ATP pools because the lowering of ATP levels under conditions of excessive PARP activation is the consequence of the increased use of NAD^+ for poly (ADP-ribose) synthesis. However, Paschen et al. (6) measured the NAD^+ and ATP levels together in a model of transient focal cerebral ischemia and reported the ATP levels to drop to 20% that of the control, whereas the NAD^+ levels remained close to the control levels. Similar results were obtained in the human umbilical vein endothelial cells after oxidant injury (19). In the present

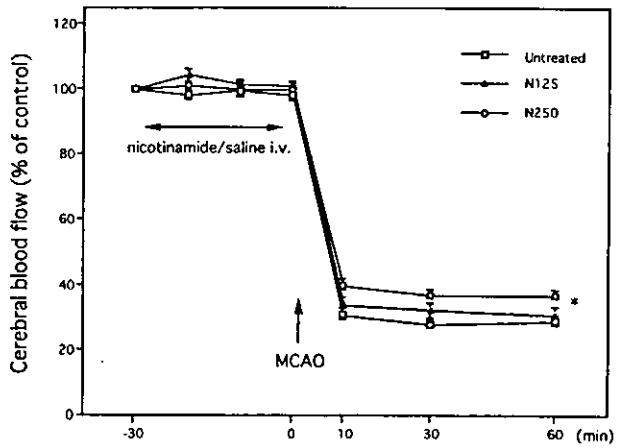

Fig. 2. The degrees of regional CBF (rCBF) reduction were compared among untreated, nicotinamide 125 mg/kg and 250 mg/kg groups in experiments 1 and 2. rCBF was measured by laser Doppler flowmetry at a point 1 mm posterior and 4 mm lateral to the bregma before and after nicotinamide injection and after MCA occlusion. The values are mean ± SEM (n = 13–14 per group). *$P < .01$ was considered to be significant for four comparisons.

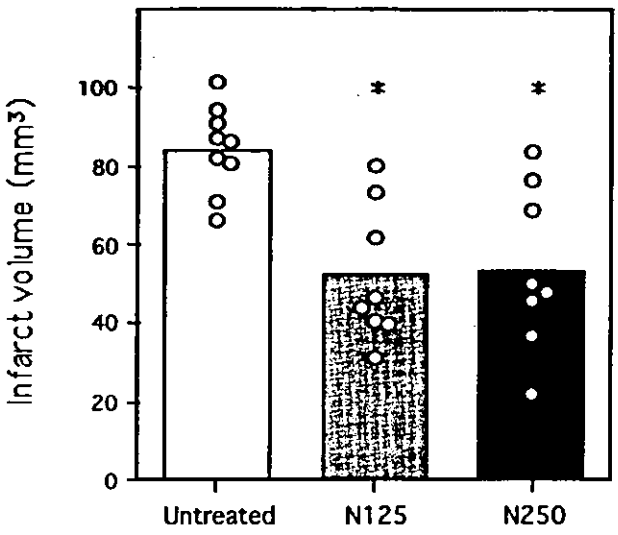

Fig. 3. Infarct volumes in the untreated, nicotinamide 125 mg/kg and 250 mg/kg groups in experiment 1. *$P < .025$ was considered to be significant for two comparisons.

study, there was no significant reduction in the NAD^+ levels in either the ischemic core or the penumbra of the untreated group, and our results were essentially the same as Paschen's findings. Hence, these findings do not support the hypothesis that a critical secondary energy failure is caused by the depletion of NAD^+ in focal cerebral ischemia. The brain NAD^+ levels increased in the contralateral non-ischemic regions (2.0- to 2.2-fold) and penumbra (1.8- to 2.0-fold) with an injection of nicotinamide 250 mg/kg, but not in the ischemic core. In the nicotinamide 125 mg/kg group, the NAD^+ levels tended to increase in the contralateral regions and penumbra, but the differences just failed to reach the conservative significance. Anyway, these results suggest that nicotinamide could be metabolized to produce NAD^+ in the penumbra where tissue viability remained for several hours after ischemic onset. Treatment with nicotinamide at a range of 7.5–15.0 mmol/L protected against NO toxicity in cultured hippocampal neurons by preventing the activation of caspase 1 and

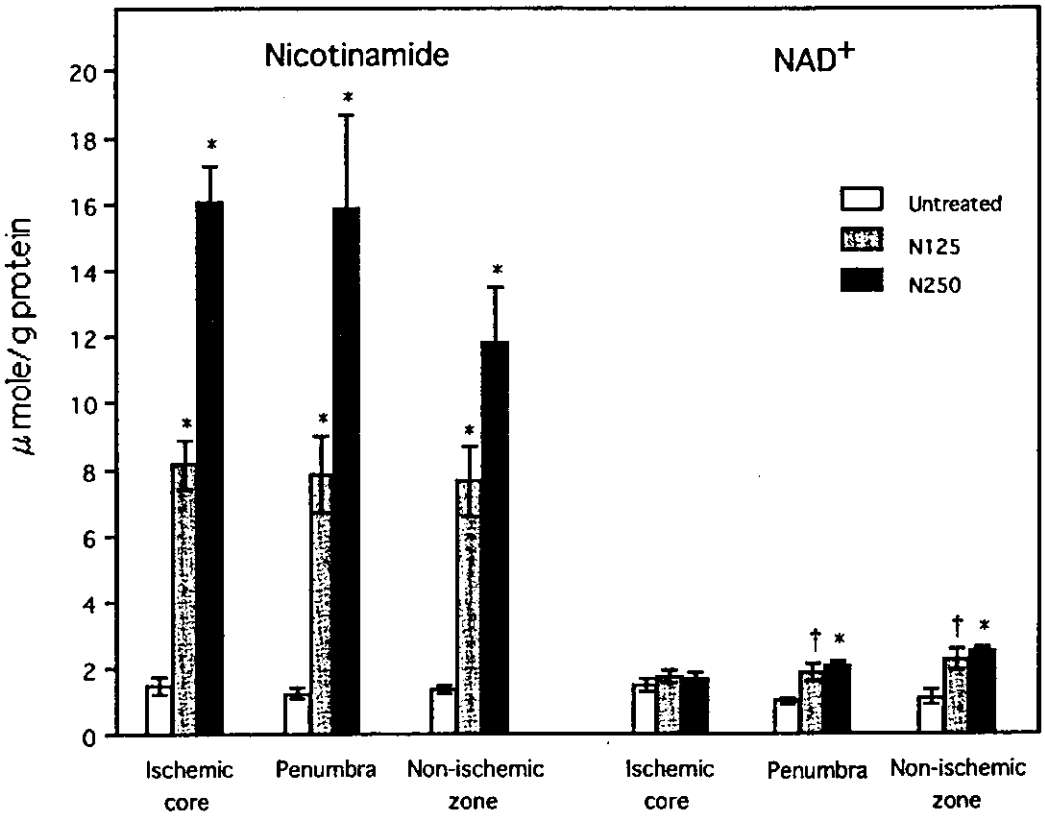

Fig. 4. Levels of nicotinamide and NAD^+ at ischemic core, penumbra, and non-ischemic zone among groups in experiment 2. Values are the mean ± SEM. †$P < .0166$, *$P < .0083$ versus control by unpaired Student's t-test. *$P < .0083$ was considered to be significant for six comparisons.

caspase3-like activities and PARP cleavage after NO exposure (7). Another *in vitro* study showed that specific inhibitors of PARP suppressed nuclear fragmentation and apoptotic body formation, which suggests that inhibition of poly(ADP-ribosyl)ation and presumed NAD^+ preservation may inhibit apoptosis (20). Therefore increased brain nicotinamide and/or NAD^+ may prevent apoptotic pathway to cell death.

Nicotinamide is known to be a potent PARP inhibitor at high doses (2–5,21,22). If nicotinamide could effectively inhibit PARP *in vivo*, the neuroprotective effect of nicotinamide observed in the present study might thus have been due to PARP inhibition. In the present study, the concentrations of brain nicotinamide remarkably increased in a dose-dependent manner in all regions examined (5.4- to 6.2-fold in 125 mg/kg groups, 8.7- to 12.4-fold in 250 mg/kg groups, compared with those in the vehicle group). The actual concentration (1.4 mM) of brain nicotinamide after 250 mg/kg of nicotinamide infusion seemed to be slightly lower than the concentration (10 mM) of nicotinamide known to be needed to sufficiently inhibit PARP. It therefore remains unclear from the present results as to whether or not the protective action of nicotinamide against ischemic brain injury is mediated through PARP inhibition.

Nicotinamide protects against oxidative damage, malonate-induced neurotoxicity, serotonin neurotoxicity, trauma, and nitric oxide toxicity (23–28). As Kamat and Devasagayam reported (25), nicotinamide concentration of 4 mM was needed to show a significant inhibition of oxidative damage in the brain mitochondria *in vitro*. Thus, again the brain level of nicotinamide in the present study seemed to be lower than that at which level nicotinamide can act as an antioxidant.

Recently, nicotinamide was used *in vivo* focal cerebral ischemia model and significantly reduced infarct volumes (8–10). Because CBF was not determined in these studies, it is not clear as to whether the beneficial effects of nicotinamide were based on a direct neuroprotection or increased regional CBF. High doses of nicotinamide (300–500 mg/kg) have been reported to increase the regional CBF and the cerebral metabolic rate of oxygen, but nicotinamide in small doses (30–50 mg/kg) demonstrated no such effect in dogs (11). In the present study the degree of regional CBF reduction did not differ between the untreated and the nicotinamide 125 mg/kg group, whereas the difference between the untreated and the nicotinamide 250 mg/kg group was significant. Although the high dose of nicotinamide increased the CBF, the infarct size was substantially attenuated even in the low-dose nicotinamide group in which the dose had no apparent vasodilative effect. These results suggest that the neuroprotective effect induced by nicotinamide is therefore independent of the vasodilative effect.

In conclusion, the infarct size was substantially attenuated even in the low-dose nicotinamide (125 mg/kg) group in which the dose had no significant vasodilative effects. This CBF independent mechanism of neuroprotection may be due to the marked increase in brain nicotinamide and, to a lesser degree, may be due to an increase in NAD^+ in the penumbra.

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Effects of carotid endarterectomy on cerebral blood flow and neuropsychological test performance in patients with high-grade carotid stenosis

Kazuhiro Kishikawa[a,b,*], Masahiro Kamouchi[a,b], Yasushi Okada[a], Tooru Inoue[c], Setsuro Ibayashi[b], Mitsuo Iida[b]

[a] *Department of Cerebrovascular Disease and Clinical Research Institute, National Kyushu Medical Center, Japan*
[b] *Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Japan*
[c] *Neurosurgery, National Kyushu Medical Center, Japan*

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Abstract

We examined the changes in cognitive function following carotid endarterectomy (CEA) in relation to the cerebral blood flow (CBF) in patients with high-grade carotid stenosis. The subjects consisted of 23 patients who underwent CEA and 17 controls matched by age and education. Single photon emission computed tomography (SPECT) and neuropsychological tests were performed 2 weeks before and 4 weeks after CEA in all patients. The preoperative CBF tests revealed a decreased vasodilatory reserve in the ipsilateral cerebral hemisphere in nine patients, which was increased after CEA. In these patients, the grade of carotid stenosis was significantly higher than in those with a normal perfusion reserve (90.2 ± 8.1% vs. 78.6 ± 11.3%, respectively, $p<0.05$). In the patient group, the postoperative scores (27.2 ± 2.9) of the mini-mental state examination (MMSE) improved significantly over the preoperative ones (26.1 ± 3.2, $p<0.05$). Moreover, the scores in the block-design test after CEA (86.8 ± 19.8) were significantly higher than those before the operation (81.8 ± 22.3, $p<0.01$). The error score in immediate retention improved from 9.0 ± 3.1 to 7.7 ± 4.0 following CEA ($p<0.05$). In the control group, none of the test scores showed significant improvement between the first and second tests. In the patients with an impaired vasodilatory reserve, the mean score of the block-design test significantly improved from 65.6 ± 22.1 to 74.0 ± 19.2 after CEA compared with those in patients without impairment ($p<0.05$). High-grade carotid stenosis was thus concluded to cause cognitive impairment due to cerebral hemodynamic failure, which is presumably reversed by CEA.

Keywords: Carotid artery; Carotid endarterectomy; Cerebral blood flow; Single photon emission computed tomography; Neuropsychological test; Cognitive function

1. Introduction

Cognitive function has been reported to be impaired in patients with occlusive carotid artery disease [1]. However, there is still little data available concerning the changes in neuropsychological functions following carotid endarterectomy (CEA). The effects of CEA on cognitive function reported have varied from study to study [2–17]. This heterogeneity might be caused by various factors including individual variation, complications due to multiple perioperative variables and the choice of neuropsychological tests. The timing of the assessment, practice and strategic responses and the type of analyses used may also affect the conclusion. As a result, the effect of CEA on neuropsychological functions remains controversial. Moreover, none of the previous studies included measurement of the cerebrovascular reserve.

Intracranial carotid lesions have been reported to be more common than extracranial ones in Japanese, while it is the opposite for Caucasians [18,19]. Although we demonstrated that extracranial atherosclerotic carotid lesions are also increasing in Japanese [20], no data is yet available concerning cognitive impairment in Japanese patients with high-grade carotid stenosis. In the present study, we exam-

* Corresponding author. Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Maidashi 3-1-1, Higashi-ku, Fukuoka 812-8582, Japan. Tel.: +81-92-642-5256; fax: +81-92-642-5271.
E-mail address: kishi-kk@cj8.so-net.ne.jp (K. Kishikawa).

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ined changes in the cerebral blood flow (CBF) and neuropsychological functions following CEA in Japanese patients with unilateral carotid stenosis to elucidate the effect of CEA on neuropsychological function.

2. Patients and methods

2.1. Patients

The subjects consisted of 23 patients who underwent CEA and 17 age-matched controls. The patients were selected from 42 consecutive patients who were admitted to our hospital and underwent CEA between 1999 and 2001. Patients having more than 60% contralateral carotid stenosis, a physical disability (Rankin disability scale>1) or a history of neuropsychological impairment due to ipsilateral cerebral infarction, were excluded from the analysis. A neurologist (Y.O.) selected 17 controls of similar age and years of education from those admitted to our hospital during the same period. They had neither carotid stenosis nor any neuropsychological impairment due to cerebral lesions. Their medical status is shown in Table 1. Brain computed tomography (CT) and/or magnetic resonance imaging (MRI) were performed on all subjects and demonstrated no significant lesions in the area of the cortical branch of cerebral arteries. The degree of carotid stenosis on angiography was assessed by the same method as that used in the NASCET study [21]. The luminal diameter at the point of greatest stenosis and at a normal part of the artery beyond the carotid bulb was measured, and the percent stenosis was determined by calculating the ratio of these two measurements. The indications for CEA were based on the guidelines of the American Heart Association [22]. CEA was performed on symptomatic patients with ipsilateral 70–99% carotid artery stenosis and unilateral carotid stenosis >50% with ulcer. All subjects gave informed consent to be examined by various neuropsychological tests.

Table 1
Demographic and clinical characteristics

	CEA group	Control group
Number	23	17
Age	68.0 ± 6.6	66.6 ± 6.8
Male/female	22:1	14:3
Degree of stenosis (%)	83.2 ± 11.5	10.6 ± 13.4
Medical status	minor stroke 13	peripheral vestibular disorder 9
	transient ischemic attack 8	syncope 5
	asymptomatic 2	transient ischemic attack 3
Risk factors		
Smoking	19	11
Hypertension	17	12
Hyperlipidemia	11	6
Diabetes	9	7
Vascular complications		
Ischemic heart disease	14	5
Arteriosclerosis obliterans	4	1

The degree of carotid stenosis was calculated using the method described by the NASCET group [21].

2.2. Neuropsychological testing

The neuropsychological tests used to evaluate the cognitive function were the mini-mental state examination (MMSE), a dementia scale (the revised version of Hasegawa's dementia scale: HDSR), a block-design test (Kohs block-design test), a word recall test (recall of 10 pairs of related words) and a visual retention test (Benton visual retention test [23,24]). These neuropsychological tests are widely employed as standard tests for Japanese patients and were used in the present study to evaluate the effects of the surgery. The MMSE and HDSR are used to assess general cognitive abilities; a full score is 30. Kohs block-design test has been reported to be significantly correlated with the Wechsler Adult Intelligence Scale and was used to evaluate intellectual performance including nonverbal composition ability, the recognition of space, general activity and self-control. For evaluation of short-term memory and verbal and visual composition ability, a word recall test and a visual retention test were used. In the Benton visual retention test, all subjects were given Form I as the initial test and Form II as the subsequent test, following the procedure of Administration A and D described in the test manual. The test performances were evaluated on the basis of the usual objective criteria and were scored in terms of either the number of correct reproductions (correct score) or the number of errors made (error score). We performed all tests in the hospital 2 weeks before and 4 weeks after CEA. These tests were performed by two speech therapists who had each examined approximately 1000 patients and were considered experienced in the administration of neuropsychological tests. The subsequent tests were done by the same therapist. All neuropsychological tests were performed in an isolated room where the patient could concentrate without difficulty. Using the same methods, we also performed these tests twice at 6 weeks interval in 17 controls.

2.3. Analysis of CBF

The cerebral circulation using single photon emission computed tomography (SPECT) was evaluated simultaneously with the neuropsychological tests. The apparatus for SPECT was the PRISM 2000XP (two-head SPECT system, Picker, USA), and the tracer was ^{99m}Tc–ethyl cysteinate dimer (ECD). The CBF in the territory of the middle cerebral artery (MCA) was quantitatively measured before

and after the intravenous administration of acetazolamide (0.017 g/kg). The baseline mean CBF was measured from a graphical analysis of the time–activity curve for the brain and aortic arch obtained from radionuclide angiography by an injection of ^{99m}Tc –ECD, and the regional CBF was calculated using Lassen's correction algorithm. Post-acetazolamide mean CBF was estimated from the baseline mean CBF, the baseline mean SPECT counts and post-acetazolamide mean SPECT counts corrected for administered dose and imaging time. The post-acetazolamide regional CBF was obtained from the post-acetazolamide mean CBF and the post-acetazolamide mean SPECT counts using Lassen's algorithm [25–27]. CBF was considered to have decreased when it was less than 80% of that in normal patients (49 ml/100 g/min). A compromised reserve was defined as a response to acetazolamide of less than 10%.

2.4. Surgical procedure

All CEA procedures were performed by a neurosurgeon (T.I.). Anesthesia was achieved by fentanyl citrate, thiamylal sodium and propofol. The blood pressure, heart rate, blood gases and various Doppler flow parameters in the carotid artery as well as an electroencephalogram were continuously monitored during the procedure. A shunt tube was inserted into both ends of the carotid artery to reserve bypass flow to the distal intracranial arteries, and an endarterectomy was carefully performed using a microscope. No complications occurred during the perioperative period. After CEA, magnetic resonance imaging of the brain was performed on all patients, and no new pathological lesions were found.

2.5. Statistical analysis

The difference in the clinical characteristics between patients and controls was analyzed by the χ-square test. We analyzed the difference in the degree of carotid stenosis in patients with and without perfusion reserve using unpaired *t*-test. The changes in the neuropsychological test scores were examined by the paired *t*-test or two-way repeated-measures ANOVA. Post hoc analysis was done by Scheffe's multiple comparison test. A *P* value exceeding 0.05 was considered to be significant. The values were expressed as the mean ± S.D.

3. Results

3.1. Demographic and clinical features of the patients

The mean ages of the patients and controls were 68.0 ± 6.6 and 66.6 ± 6.8 years, respectively. The average degree of stenosis was 83.2 ± 11.5%. Table 1 summarizes the clinical characteristics of the patients and controls. No significant difference in age and sex was detected between the groups. The rate of concomitant risk factors including smoking, hypertension, hyperlipidemia and diabetes mellitus was similar between patients and controls.

3.2. CBF and carotid stenosis

The resting state CBF was decreased in 15 patients (65%). Of these patients, the decreased perfusion in the ipsilateral MCA territory in a resting state improved in seven patients after CEA. The perfusion reserve was decreased in nine patients (39%) before CEA, all of whom showed improvement after CEA. The average CBF within the territory of the ipsilateral MCA in a resting state was 33.5 ± 5.5 ml/100 g/min before CEA and 36.0 ± 3.6 ml/100 g/min after surgery. The average CBF measured after the administration of acetazolamide was 40.9 ± 8.9 ml/100 g/min and 46.9 ± 5.7 ml/100 g/min before and after CEA, respectively. On the contralateral side, pre- and postoperative CBF in the MCA territory were 39.6 ± 4.2 ml/100 g/min and 40.4 ± 5.7 ml/100 g/min at rest, and they were 48.6 ± 8.4 ml/100 g/min and 50.3 ± 6.0 ml/100 g/min, respectively, after the administration of acetazolamide. We divided the patients into two groups according to the condition of the CBF, i.e., patients with a normal vasodilatory reserve (group 1) and those with a decreased reserve (group 2). Fig. 1 shows the distribution of the degree of carotid stenosis in groups 1 and 2. Stenosis was significantly more severe in group 2 (90.2 ± 8.1%) than in group 1 (78.6 ± 11.3%, $p < 0.05$). Moreover, all patients in group 2 had more than 70% carotid stenosis.

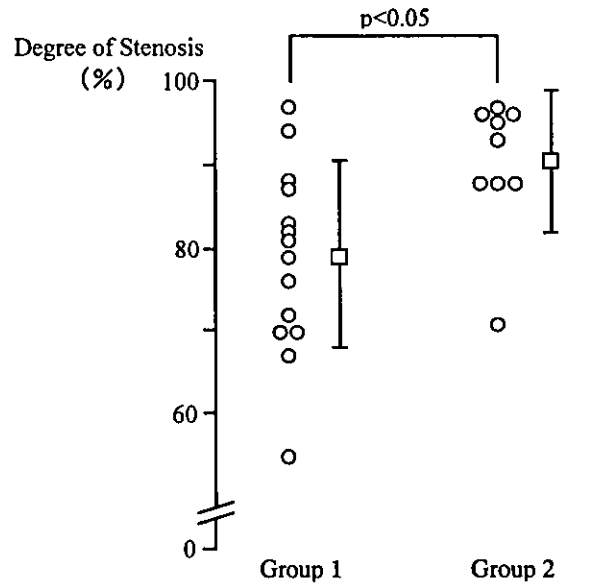

Fig. 1. Carotid stenosis in patients with preserved and decreased perfusion reserve. The grade of carotid stenosis was plotted in patients with preserved (group 1) and decreased vasodilatory reserve (group 2). Squares show the average degree of carotid stenosis in both groups. Error bars indicate the S.D. from the mean. The difference between the groups was analyzed by unpaired *t*-test.

Table 2
Neuropsychological test performances before and after carotid endarterectomy

Tests	Before CEA	After CEA
MMSE	26.1 ± 3.2	27.2 ± 2.9*
HDSR	25.9 ± 4.2	26.8 ± 3.4
Block-design test	81.8 ± 22.3	86.8 ± 19.8**
Verbal memory test	9.3 ± 1.6	9.6 ± 1.2
Visual retention test		
Immediate memory		
Correct	4.8 ± 1.6	5.2 ± 1.8
Error	9.0 ± 3.1	7.7 ± 4.0*
Delayed memory		
Correct	3.7 ± 1.2	4.4 ± 1.6**
Error	12.1 ± 5.1	10.5 ± 5.2

The pre- and postoperative test scores were compared using paired *t*-test. Values are mean ± S.D. *P* values exceeding 0.05 were considered to be significant.
*$p<0.05$.
**$p<0.01$.

3.3. Changes in the neuropsychological test results following CEA

Neuropsychological tests were performed on all patients (Table 2). The average score of the HDSR before and after CEA was 25.9 ± 4.2 and 26.8 ± 3.4, respectively. The score of the word recall test did not show a significant improvement either (9.3 ± 1.6 and 9.6 ± 1.2, before and after CEA, respectively). On the other hand, the postoperative MMSE score (27.2 ± 2.9) did improve significantly over the preoperative one (26.1 ± 3.2, $p<0.05$). Moreover, the scores on the block-design test after CEA (86.8 ± 19.8) were significantly higher than those before the operation (81.8 ± 22.3, $p<0.01$). The average number of correctly reproduced designs (correct score) in immediate retention before and after CEA was 4.8 ± 1.6 and 5.2 ± 1.8, and the number of errors (error score) in delayed retention before and after CEA was 12.1 ± 5.1 and 10.5 ± 5.2, respectively, which was not significant. However, the total number of errors in immediate retention improved from 9.0 ± 3.1 to 7.7 ± 4.0 following CEA ($p<0.05$). The mean of correct scores in the delayed retention test before CEA (3.7 ± 1.2) also significantly improved after CEA (4.4 ± 1.6, $p<0.01$).

In the control group, all test scores were comparable to the preoperative scores in the patient group. No significant differences were seen between the first and the second tests in the control group (Table 3).

Table 3
Neuropsychological test performances in controls

Tests	First	Second
MMSE	26.4 ± 3.8	26.6 ± 4.0
HDSR	26.1 ± 3.5	26.2 ± 4.4
Block-design test	83.1 ± 21.9	85.7 ± 20.7
Verbal memory test	9.1 ± 1.9	9.4 ± 1.7
Visual retention test		
Immediate memory		
Correct	5.1 ± 1.9	5.4 ± 2.0
Error	7.9 ± 3.6	7.1 ± 4.5
Delayed memory		
Correct	4.2 ± 1.9	4.3 ± 1.3
Error	10.9 ± 4.2	10.7 ± 3.8

First and second test scores were compared using paired *t*-test. *P* values exceeding 0.05 were considered to be significant.

Table 4
Change in block-design test score in group 1 and group 2

Perfusion reserve	Degree of stenosis (%)	Before CEA	After CEA
Group 1 (14)	78.6 ± 11.3	92.2 ± 15.7	95.1 ± 15.7
Group 2 (9)	90.2 ± 8.1	65.6 ± 22.1	74.0 ± 19.2

The values in parenthesis indicate the number of patients. Interactions among block-design test score, pre-/post-CEA status and groups 1/2 are analyzed using ANOVA. Post hoc analysis was done by Scheffe's multiple comparison test. The interaction between the block-design test score and pre-/post-CEA was statistically significant ($p<0.05$). The scores of the block-design test were significantly different in the groups ($p<0.01$) and in pre- and post-CEA conditions ($p<0.01$).

3.4. Change in the block-design test results in normal and decreased perfusion reserve

To examine whether the improvement depended on the condition of the CBF, the change in the scores of the block-design test, MMSE and visual retention test were further analyzed according to the cerebral hemodynamic condition (Table 4). The difference in the test scores between group 1 and group 2 was examined. The score on the block-design test of group 2 was significantly lower than that of group 1 ($p<0.01$). The time course of the block-design test score was significantly different between the groups ($p<0.05$). No significant difference in the improvement of the scores of the MMSE and visual retention test was detected in two groups. We also compared the scores before and after CEA depending on the affected side. However, whether the affected side was the right or left side had no significant difference in the score.

4. Discussion

4.1. Cerebral hemodynamics in carotid stenosis

Hemodynamic failure is defined as a fall in the cerebral perfusion pressure below the lower threshold of cerebral autoregulation. Stenosis of the carotid artery is involved in distal hemodynamic failure by lowering the cerebral perfusion pressure. The blood flow may be affected by various factors, including the cross-sectional area, length of the stenosis, flow velocity and blood viscosity. The most significant factor in carotid stenosis is the cross-sectional area. A previous report showed that 40% and 64% reductions in

blood flow resulted from stenosis of 75% and 84% in diameter, respectively [28]. In the present study, the grade of stenosis was significantly higher (90.2 ± 8.1%) in patients with a decreased vasodilatory reserve (Fig. 1). In these patients, the vasodilatory response demonstrated by SPECT recovered following CEA. Vanninen et al. [29] showed a significant increase in the blood flow in the ipsilateral carotid artery following endarterectomy using magnetic resonance phase-contrast flow quantification. The mean internal carotid artery flow measured by a square wave electromagnetic flowmeter has been shown to increase from 133 to 212 ml/min on completion of CEA [30]. The removal of stenosis might thus drastically improve insufficient CBF.

4.2. Neuropsychological changes following CEA

In the present study, there was no significant change in HDSR or word recall test results, although a significant improvement was seen in the postoperative scores in the MMSE, block-design and visual retention tests. Although it is still unknown whether the effect is long lasting or not, the results shown in the present study are consistent with those indicating a beneficial effect of CEA on cognitive function.

Although the extent to which CEA affects cognitive function has been the subject of a number of studies, the conclusions are still conflicting [31]. Williams and McGee [2] first evaluated comprehensive psychological studies of six patients with carotid artery occlusion and observed no significant improvement in the psychological status after endarterectomy. Similar reports indicated that no significant difference was found in the postoperative cognitive scores when compared with the patients' preoperative scores or with the scores of a control group matched by age and years of education [2–8]. On the other hand, other reports suggested that CEA improves certain aspects of intellectual performance [9–15]. A recent comprehensive review noted that in 16/28 studies, the authors concluded that CEA leads to improved cognitive test performance, while 12/28 studies found that CEA had either no beneficial effect or led to a deterioration in cognition. As a result, the findings concerning the effects of CEA on the neuropsychological functions vary depending on the reports [31]. The studies were found to differ in many methodological factors, e.g., sample size ($n = 6–145$), timing of postoperative assessment (1–104 weeks), type of patient (transient ischemic attacks and strokes) and control group, severity and side of carotid stenosis and the range of cognitive tests (5–34 tests) [31].

In the present study, the neuropsychological tests were performed twice at an interval of 6 weeks. To determine whether a practice effect affected the result, we examined controls matched by age and educational level who did not undergo CEA twice at an identical interval. However, no significant differences were detected between the first and the second tests in the control group. Therefore, we concluded that the changes seen in the CEA group were greater than one would expect. To rule out the possible involvement of neurological symptoms and the side of the carotid stenosis, we additionally investigated the influence of these factors on the impact of CEA on cognitive function. We separately analyzed the change in the cognitive function between the symptomatic and asymptomatic patients. In 21 symptomatic patients, the trends in improvement were essentially the same as the overall results (data not shown). Moreover, whether the affected side was the right or left side produced no significant difference in the scores of the patients. Although it is still possible that the effect of CEA on cognitive function is underestimated due to the sensitivity of the tests as well as the ceiling effect, the present results support the idea that CEA improves cognitive function.

4.3. CBF and neuropsychological change

None of the studies investigating the effect of CEA on the cognitive function included measurements of the cerebrovascular reserve. To elucidate the possibility that the change in CBF is involved in the effect of CEA on cognitive function, we further analyzed the effect of cerebral hemodynamics on cognitive function and obtained the new insight into its effect. In the present study, the 23 subjects undergoing CEA were divided into two groups according to their cerebral perfusion status, and the change in the neuropsychological test performance was compared between the groups. As a result, the test scores were significantly lower in the group with hemodynamic failure compared to that without. Moreover, the difference in the time course of the block-design test score was statistically significant between the group with and without vasodilatory reserve. These results indicate that the neuropsychological test performance might improve due to an increased CBF. In a previous report, Whitten et al. [32] defined the low-flow-endangered brain as having an angiographically demonstrable 75% or greater reduction in the cross-sectional area of the internal carotid artery. Moreover, the patients with low-flow-endangered brains were shown to have significantly greater improvements in memory and mental abilities after CEA than did the control group [9]. A recent report also showed that neuropsychological deficits are correlated with reduced functional positron emission tomography/SPECT parameters (regional CBF/regional glucose metabolism) but not with the severity of white matter lesions on MRI in patients with cerebral microangiopathy [33]. The present results may be consistent with these results and thus support the hypothesis that CEA improves CBF by removing stenosis and, consequently, neuropsychological impairments. However, the pre–post-improvements in cognitive measures for the patients appear to be slight. In addition, we reanalyzed the pre–post/patient–control interaction effect among each neuropsychological test using two-way repeated-measures ANOVA instead of paired *t*-test. As a result, some of the

significant results dropped out (data not shown). Larger trials or meta-analyses would be required to exclude the possibility that the difference might be within the range of the measurement error of the tests.

In conclusion, high-grade carotid stenosis might induce a cognitive impairment due to cerebral hemodynamic failure. This impairment is presumably reversed by CEA.

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Photothrombotic Middle Cerebral Artery Occlusion and Reperfusion Laser System in Spontaneously Hypertensive Rats

Hiroshi Yao, MD; Hiroshi Sugimori, MD; Kenji Fukuda, MD; Junichi Takada, MD; Hiroaki Ooboshi, MD; Takanari Kitazono, MD; Setsuro Ibayashi, MD; Mitsuo Iida, MD

Background and Purpose—To establish a less invasive and reproducible focal ischemia model in the rat, we adopted a 2-laser system (ie, photothrombosis and YAG laser–induced reperfusion).

Methods—The distal middle cerebral artery (MCA) of spontaneously hypertensive rats was occluded by 568-nm krypton laser and intravenous infusion of the photosensitizing dye rose bengal and was recanalized by 355-nm ultraviolet laser irradiation. Cerebral blood flow was determined by laser-Doppler flowmetry at the penumbral cortex. Infarct volume was determined at 3 days after distal MCA occlusion.

Results—Brain temperature determined with infrared thermography was maintained within an acceptable range of approximately 1°C upper shift of the center of brain temperature distribution during krypton or YAG laser irradiation. The average of the values (23 experiments; n=163) of coefficient of variation of infarct volume was 21±6%, indicating high reproducibility of this model. After distal MCA occlusion, cerebral blood flow was decreased to 32±16% of the control values and was increased to 98±21% after YAG laser–induced reperfusion. Infarct volume in these rats was 61±18 mm^3 (coefficient of variation=30%; n=6).

Conclusions—We have characterized a highly reproducible focal ischemia model utilizing a 2-laser system, one to induce thrombotic MCA occlusion and the other to facilitate reperfusion. (***Stroke.*** **2003;34:2716-2721.)**

Key Words: cerebral ischemia, focal ■ photochemistry ■ reperfusion injury ■ thrombolysis ■ rats

To elucidate the pathophysiology of ischemic stroke and to find potential neuroprotective strategies, validated animal models of focal cerebral ischemia are indispensable. The constant objection against animal studies is the failure of pharmacological studies to translate from the animal model to the clinical setting. Nevertheless, the relevance of animal stroke models is reasonably evident, as discussed by Ginsberg.[1] In particular, rodent models have crucial advantages of lower cost, suitability for physiological monitoring, reproducibility of lesion size, and ease of conducting replicate studies. Although Huang et al[2] suggested that the number of animals needed to demonstrate a 50% decrease in infarction volume may be reduced by 41% in their modified transorbital baboon model compared with a previous nonhuman primate stroke model, the coefficient of variation (CV) for infarct volume was still considerably large (62%).

Tamura et al[3] first established the proximal middle cerebral artery (MCA) occlusion model in the rat. Subsequently, distal MCA occlusion methods were developed by Chen et al[4] and Brint et al.[5] Recently, the intraluminal suture method has been widely used not only in rats but also in mice.[6–10] However, proximal and distal MCA occlusion procedures are surgically demanding and may induce local traumatic effects, and, in the suture model, the success rate of occlusion and reproducibility of infarct size are sometimes unsatisfactory. Furthermore, the intracranial suture method is proposed to be an internal carotid artery occlusion model rather than a pure MCA occlusion model.[11]

A photothrombotic distal MCA occlusion model in spontaneously hypertensive rats (SHR) yields a highly reproducible infarct volume and does not entail extensive surgery or opening of the dura, thereby avoiding local tissue trauma at the site of MCA occlusion.[12,13] This model encompasses appropriate physiological monitoring, associated risk factors for stroke (ie, hypertension and aging), and clinically relevant pathophysiology of thrombosis. A recent immense advance in the practice of the photothrombotic stroke method in the rat achieved by Watson et al[14] is the ultraviolet laser–induced reperfusion method. In the present study we discuss the usefulness of laser methods in establishing stroke models in SHR.

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From the Department of Medicine and Clinical Science, Second Department of Internal Medicine, Graduate School of Medical Sciences, Kyushu University, Fukuoka (H.Y., H.S., K.F., J.T., H.O., T.K., S.I., M.I.), and Laboratory for Neurochemistry, Hizen National Hospital, Saga (H.Y., K.F.), Japan.

Correspondence to Hiroshi Yao, MD, Department of Medicine and Clinical Science, Second Department of Internal Medicine, Graduate School of Medical Sciences, Kyushu University, Maidashi 3-1-1, Higashi-ku, Fukuoka 812-8582, Japan. E-mail yao-h@hizen2.hosp.go.jp

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Materials and Methods

All procedures were done in accordance with the Animal Care Guidelines at Kyushu University and the Law (No. 105) and Notification (No. 6) of the Japanese Government.

Surgical Preparation

SHR/Kyushu (aged 5 to 7 months), maintained in the Kyushu University Animal Center under specific pathogen-free conditions, were used in this study. SHR/Izm, stroke-prone SHR (SHR-SP)/Izm, Wistar-Kyoto (WKY)/Izm, and Sprague-Dawley rats were obtained from commercial vendors at the age of 3 months and used at the age of 5 to 7 months. Rats were anesthetized with halothane (3% for induction, 1.5% during the surgical preparation with a face mask, 0.75% after intubation, and 0.5% for maintenance) in a mixture of 70% nitrous oxide and 30% oxygen. The right femoral artery and vein were cannulated with the use of PE 50 tubing. The rats were endotracheally intubated with PE 240 tubing for male rats or PE 205 tubing for female rats. Pancuronium bromide (an initial dose of 0.3 mg followed by 0.1 mg every 30 minutes) was intravenously injected, and the rats were mechanically ventilated. Mean arterial blood pressure was continuously monitored. Physiological variables were maintained within normal range.

Rats were mounted on a stereotaxic head holder in the prone position, and a 2-cm incision was made vertically midway between the right orbit and the right external auditory canal. The temporalis muscle was separated and retracted, and a burr hole 3 mm in diameter was made 1 mm rostral to the anterior junction of the zygoma and squamosal bone under an operating microscope (OPMI 111, Carl Zeiss), revealing the distal segment of the MCA above the rhinal fissure. A thin bone layer was preserved to prevent injury to the brain and was carefully removed with forceps. The dura was thereby left intact.

To evaluate mechanical stress at the irradiation or occlusion site, we used 10 SHR: transient (30- or 60-minute) distal MCA occlusion by the laser method (n=2) or by a microclip (n=2) and then immediate perfusion-fixation after reperfusion; krypton and/or YAG laser irradiation without rose bengal infusion (n=3) and perfusion-fixation after 3 days of survival; and staining with TTC 3 days after either craniectomy only, clip sham occlusion, or krypton laser followed by YAG laser irradiation without rose bengal infusion (n=3).

Photothrombotic MCA Occlusion

A krypton laser operating at 568 nm (Innova 301, Coherent Inc, or 643-Y-A01, Melles Griot Inc) was used to irradiate the distal MCA at a power of 20 mW for 4 minutes. The laser beam was focused with a 30-cm focal length convex lens (KPX 112, Newport Corporation) and positioned with a mirror onto the distal MCA. The photosensitizing dye rose bengal (15 mg/mL in 0.9% saline; Wako Pure Chemical Industries Ltd) was administered intravenously at a body dose of 20 mg/kg over 90 seconds starting simultaneously with 4 minutes of laser irradiation. For permanent occlusion without corresponding counterpart groups being subjected to reperfusion of the occluded MCA, an elliptical, almost linear laser beam (linear hit)

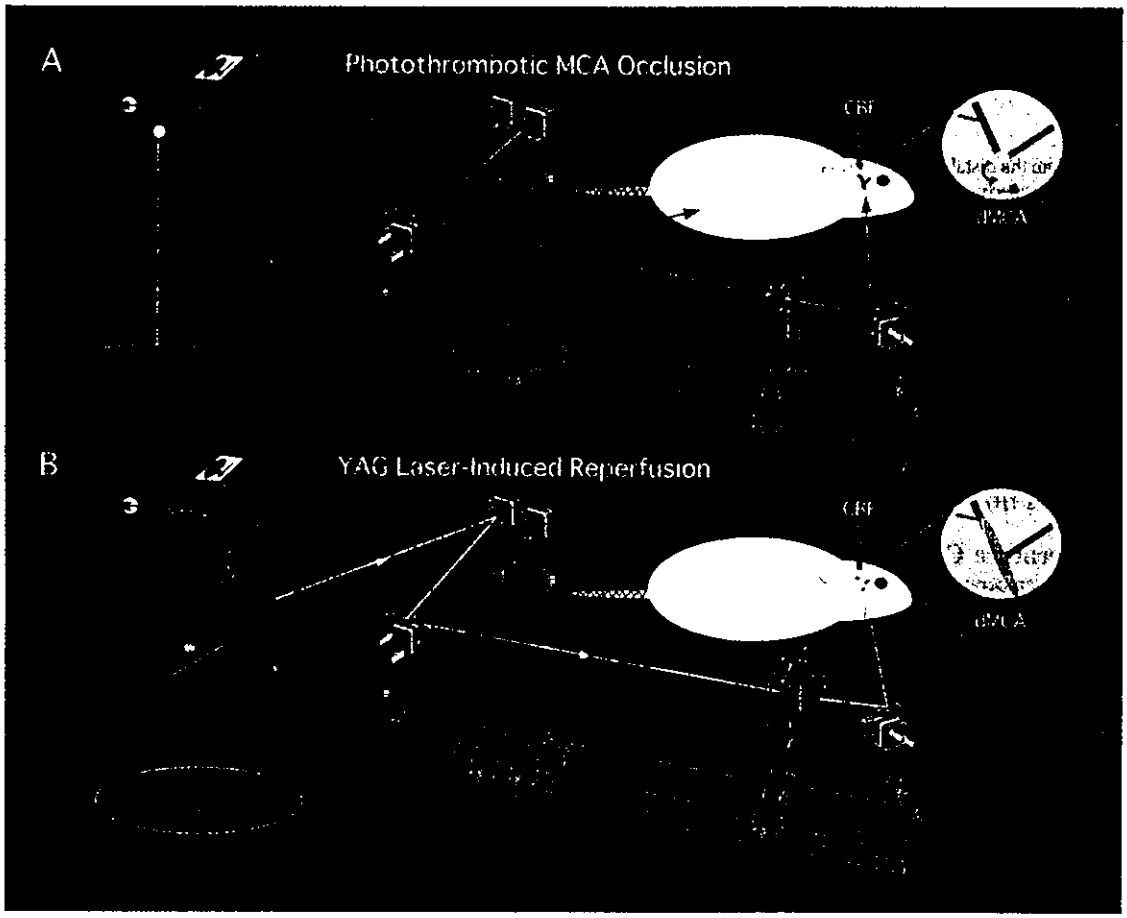

Figure 1. A, For thrombotic distal MCA (dMCA) occlusion, a krypton laser operating at 568 nm was used to irradiate the distal MCA. The photosensitizing dye rose bengal was administered intravenously at a body dose of 20 mg/kg over 90 seconds starting simultaneously with 4 minutes of laser irradiation. B, After MCA occlusion, a Q-switched, frequency-tripled YAG laser operating at 355 nm (16 mW, 15 Hz, average power 2.3 W/cm^2) was focused with a 30-cm focal length cylindrical lens and positioned with a mirror enveloping the occluded distal MCA.