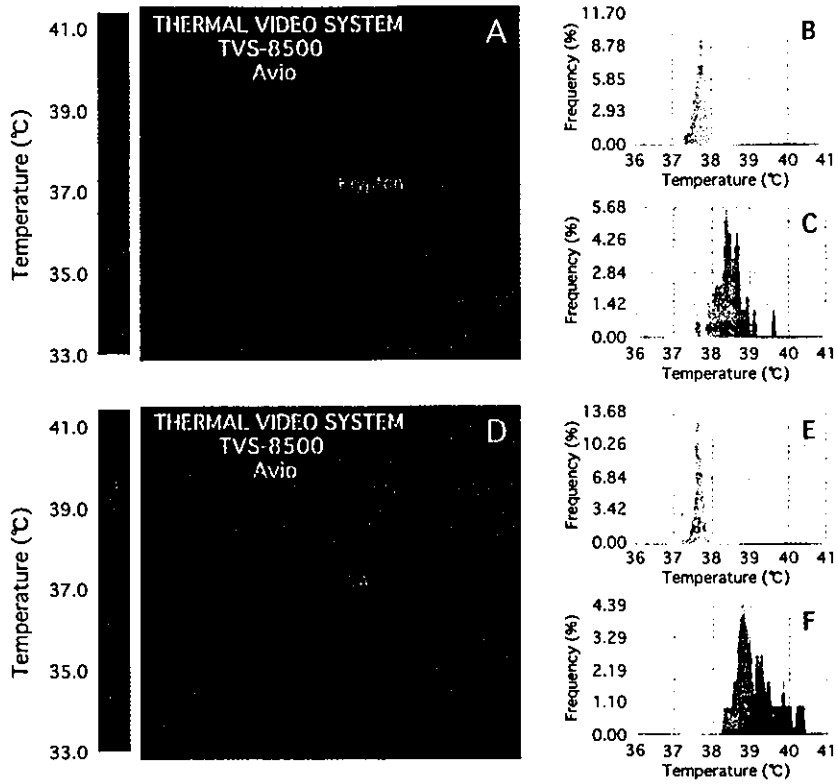

Figure 2. Brain temperature determined with infrared thermography equipment before (B, E) and at 3 minutes of krypton (A, C) or YAG (D, F) laser irradiation. B, C, E, and F, Frequency of cortical temperature distribution within the regions of interest indicated by circles in A and D.

was used to irradiate the distal MCA, as previously described.[12,13] In the case of subsequent reperfusion, a round laser beam was focused at the Y-shaped juncture of the frontal and parietal branches for 2 minutes, and then the laser beam was moved to an additional site just proximal to the first irradiated site for 2 minutes (2-point hit) (Figure 1A).

Reperfusion by YAG/Neodymium Laser Irradiation

At certain times after MCA occlusion, a Q-switched, frequency-tripled YAG/neodymium laser operating at 355 nm (16 mW, 15 Hz, average power 2.3 W/cm^2) (Minilite II, Continuum Inc) was focused with a 30-cm focal length cylindrical lens (CKX 300, Newport Corporation) and positioned with a mirror enveloping the occluded distal MCA (Figure 1B).

Brain Temperature Control

Rectal and head temperatures were maintained at approximately 37.5°C and 36.5°C, respectively, by means of a warming lamp. In addition to the routine head temperature monitoring with a thermocouple probe, changes in brain temperature were determined in 2 male SHR/Kyushu with an infrared thermography system (TVS-8500, Avio System Technology Co, Ltd), in which temperature sensitivity is 0.025°C (30°C black body) and the spatial resolution is approximately 0.2 mm (Figure 2A and 2D). Brain temperature 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, as shown in Figure 2.

Measurement of Regional Cerebral Blood Flow

In 6 male SHR/Kyushu subjected to 2 hours of transient MCA occlusion, changes in regional cerebral blood flow (CBF) were measured at 1 mm posterior and 4 mm lateral to the bregma with laser-Doppler flowmetry (ALF 21D, Advance Co Ltd). Changes in CBF were expressed as a percentage of the average of 2 or 3 baseline values. In 3 male and 5 female SHR/Kyushu, regional CBF was measured by laser-Doppler flowmetry at points 1 mm posterior and 2.0, 2.5, 3.0, 3.5, and 4.0 mm lateral to the bregma (Figure 3A) by scanning the laser-Doppler probe with a stereotaxic device before and 30 minutes after distal MCA occlusion. The distance of the infarct rim from the midline was determined on 2,3,5-triphenyltetrazolium chloride (TTC)–stained sections with National Institutes of Health Image software (version 1.56) (Figure 3B). Then the infarct rim distance was plotted against CBF, as described in Figure 3C.

Infarct Volume

Three days after ischemic insult, rats were decapitated under amobarbital anesthesia (100 mg/kg), and brains were rapidly removed. The brain was cooled in ice-cold saline for 10 minutes and was cut into 2-mm-thick coronal sections in a cutting block. Then the brain slices were stained with TTC (Wako Pure Chemical Industries Ltd) at 37°C for 30 minutes in the dark. Infarct volume was calculated by the trapezoidal rule with National Institutes of Health Image software (version 1.56), as previously described.[13]

Eight male hypertensive rats were used for a comparative analysis between TTC and hematoxylin-eosin methods to determine the extent of infarction produced by MCA occlusion. A TTC-stained coronal slice, made at 6 mm from the frontal pole, was photographed and then stained with hematoxylin-eosin.

Reproducibility of this model was investigated by reviewing all the experiments (sham-operated or control data) done in our laboratory during 1995–2001 (Table). The surgeon-1 experiment was the first experiment for each researcher done after experiments on several (usually 5) practice rats.

Statistical Analysis

Values are mean±SD. The statistical differences in infarct area expressed as a percentage of hemispheric area between TTC and hematoxylin-eosin groups were determined by paired *t* test and linear regression analysis. Statistical power was evaluated according to Cohen.[15]

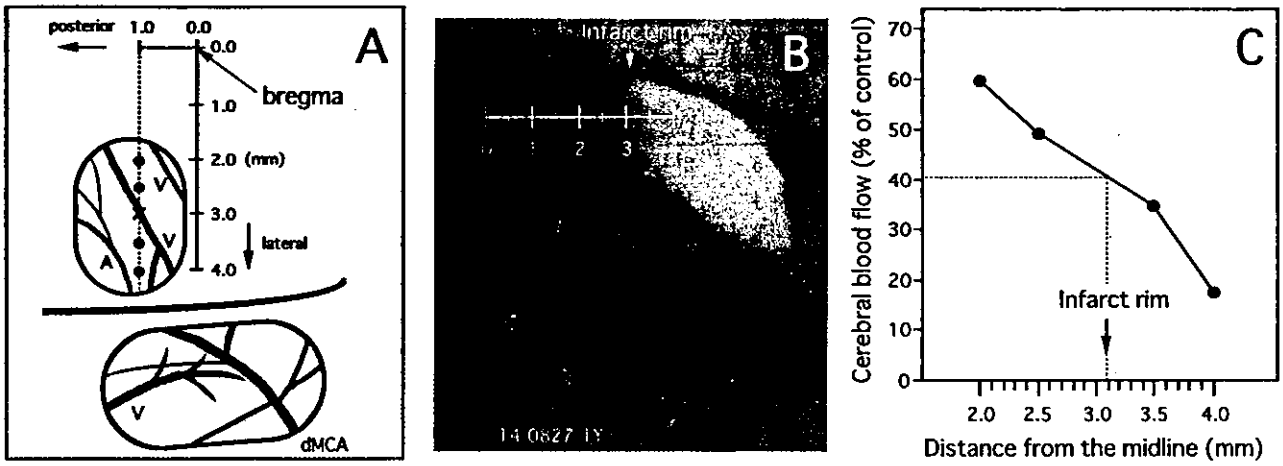

Figure 3. A, Schematic representation of points of CBF measurements. By scanning laser-Doppler probe, CBF was determined at points 1 mm posterior and 2.0, 2.5, 3.0, 3.5, and 4.0 mm lateral to the bregma. B, Distance of infarct rim from midline was determined on TTC-stained sections at 3 days. C, Infarct rim distance was plotted against CBF, which provides early ischemic CBF at later infarct rim (ie, CBF threshold for infarction). dMCA indicates distal MCA.

Results

Effects of Mechanical Stress on Craniectomy Site

Figure 4 demonstrates the effects of craniectomy, laser irradiation, or a microclip on cortical surface morphology and MCA. Craniectomy alone produced a small necrotic lesion on the cortical surface, which was seen superior to the irradiation site (Figure 4D). On TTC-stained section, the effects of craniectomy were not remarkable, and no lesion was observed after laser irradiation (Figure 4A and 4C). However, clip sham occlusion produced a small necrotic lesion (Figure 4B) and a small amount of subarachnoid hemorrhage around the distal MCA (Figure 4F).

MCA Occlusion and Reperfusion

The irradiated MCA was completely occluded by an intraluminal thrombus within 4 minutes after simultaneous laser irradiation and rose bengal infusion, which was confirmed through the operating microscope. At approximately 1 minute of YAG laser irradiation, small streaks of blood penetrated into thrombus and gradually increased in volume, and then the entire thrombus disappeared within 3 minutes (Figure 5). In our experience, only 1 rat among a series of 26 SHR/Kyushu showed an exceptionally low major branching point of the MCA, which made YAG laser irradiation onto the entire length of occluded artery impossible.

Changes in Penumbral CBF

In 6 male SHR/Kyushu subjected to 2 hours of transient MCA occlusion, CBF was decreased to 32±16% of the control values after distal MCA occlusion for 10 minutes and was stable thereafter. After YAG laser–induced reperfusion, CBF increased to 98±21%. Infarct volume in these rats was 61±18 mm^3 (CV=30%).

In 1 male SHR/Kyushu subjected to permanent MCA occlusion, the distance of the infarct rim from midline was 3.1 mm (Figure 3B), which indicated that the flow threshold for infarction was 41% of the resting CBF (Figure 3C). Male SHR/Kyushu showed a flow threshold of 43±5% of the resting CBF. In 5 female SHR/Kyushu, the flow threshold for infarction was calculated as 32±5% of the resting CBF.

Reliability and Reproducibility of Infarct Volume

The size of infarct area was 23.1±1.9% on TTC-stained slices, which was not different from the value of 23.8±2.5% on hematoxylin-eosin–stained sections (P=0.207, paired t test). Linear regression analysis revealed a good correlation between the 2 methods, where r=0.816, slope=1.054, and y intercept=−0.550%, which is significantly different from a line with slope=0 (P=0.0134) and is not significantly different from a y intercept=0 (P=0.9404). Thus, TTC staining was a reliable indicator of 3-day-old infarction.

An average of the values of CV of infarct volume was 21±6%, indicating high reproducibility for this model (Table). Therefore, in case of the 30% differences in infarct volume between the groups (eg, 30% reduction in infarct volume after treatment), the effect size d is 1.43 (30%/21%). The relative seriousness of type I to type II error is considered to be 0.05/0.20. Then, from Table 2.4.1 by Cohen,[15] for significance criterion a2=0.05 for 2-tailed test, effect size d=1.40, and row power=0.80, the number of 9 rats in each group is enough to exclude type II error.

Discussion

We adopted the photothrombotic strategy instead of mechanical occlusion of the MCA because the former does not entail opening the dura. Although any model requiring craniectomy necessitates a good deal of skill, the advantages of this method include relatively slight invasiveness, negligible incidence of mortality, and a high degree of reproducibility. In case of mechanical occlusion, for example, an unexpected finding of Glazier et al[16] was cortical expression of heat shock protein 72 and induction of ischemic tolerance after sham occlusion of the distal MCA, indicating substantial stress to the cortex even in sham-occlusion rats. Scanning electron micrography of luminal MCA showed that the irradiated segment of the MCA

Reproducibility of Infarct Size

Surgeon	Strain		Infarct Volume, mm³* Mean±SD	n	CV
Y-1	SHR/Kyushu	M	85±17	6	21
	Sprague-Dawley	M	12±2	4	19
Y-2	SHR/Kyushu	M	93±23	8	25
T-1	SHR/Kyushu	M	95±20	12	22
T-2	SHR/Kyushu	M	116±27	8	23
T-3	SHR/Kyushu	F	58±14	8	25
	SHR/Kyushu†	F	88±16	8	18
C-1	SHR/Kyushu	M	84±12	8	14
	SHR/Izm	M	62±11	10	17
	SHR/Kyushu	F	59±9	8	16
	SHR/Izm	F	35±8	5	23
	WKY/Izm	M	23±4	6	16
C-2	SHR/Izm	M	63±12	7	19
	SHR/Izm	M	56±6	7	11
0	SHR/Kyushu	M	70±16	5	22
F	SHR/Kyushu	F	61±16	5	27
	SHR/Kyushu	F	55±16	5	29
K-1	SHR/Kyushu	M	115±38	10	33
K-2	SHR/Kyushu	M	101±18	7	18
S	SHR/Kyushu	M	84±11	9	13
Y-3	SHR/Kyushu	M	100±31	5	31
	SHR-SP/Izm	M	97±25	6	26
Y-4‡	SHR/Kyushu	M	82±18	6	22

Coefficient of variation (CV) = SD/mean. M, male; F, female
*Not subjected to reperfusion.
†Female aged SHR/Kyushu.
‡2-point hit.

appeared relatively normal, whereas the endothelial layer was damaged at the clipped site.[17,18] Although the suture method has the advantage of not requiring craniectomy with its associated surgical trauma, mortality was high in some studies,[19] or the survival time was short, and subarachnoid hemorrhage may occur because of perforation of intracranial arteries.[20]

Koizumi's method of MCA occlusion by intraluminal suture,[6] subsequently modified by Longa et al,[7] gained popularity in stroke research owing to the relative simplicity of the surgical procedure. By coating the suture with poly-L-lysine, a polycationic substance thought to make the suture more adherent to the vascular endothelium, Belayev et al[10] showed a much more consistent infarction (CV=38% and 8%). In our photothrombotic MCA occlusion model, all rats develop infarction after

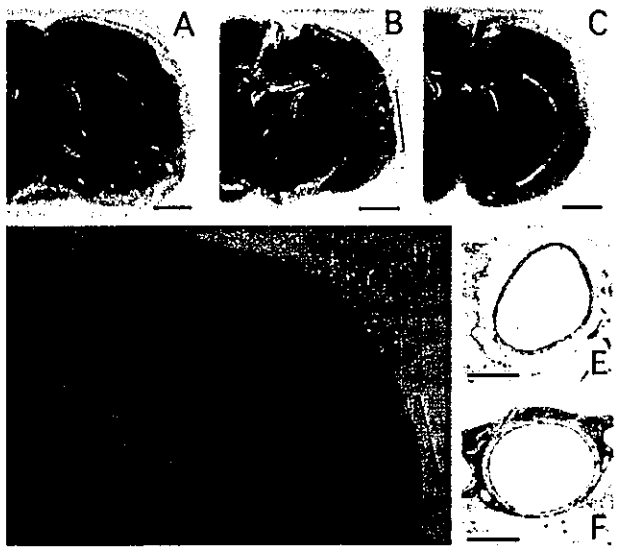

Figure 4. A to C, TTC-stained section of craniectomy only (A), clip sham occlusion (B), and krypton laser irradiation (20 mW, 4 minutes) followed by YAG laser irradiation (16 mW, 3 minutes). Bar=2 mm. B, Clip sham occlusion caused a necrotic lesion (bracket). D, Hematoxylin-eosin–stained coronal section shows a necrotic lesion (arrowheads) by craniectomy. Bracket indicates irradiation site; RF, rhinal fissure. Bar=1 mm. E and F, Distal MCA photochemically occluded for 30 minutes followed by YAG laser–induced reperfusion (E) or occluded by a microclip for 30 minutes (F). F, A small amount of subarachnoid hemorrhage around the distal MCA was observed (asterisks). Bar=100 μm.

krypton laser irradiation and rose bengal infusion with highly reproducible infarct volumes (average CV=21%). This photothrombotic model permits a reduction in the number of animals needed to establish the efficacy of a therapeutic agent.

The basis of this photothrombosis model is functional endothelial damage stimulated by an intravascular photochemical reaction, which results in a specific platelet-based response to cerebral vessel damage.[20] Pathological phenomena unique to thrombotic stroke have been observed: thrombogenically activated blood leads to the formation of blood-borne factors, which causes detrimental effects on blood-brain barrier and on ische-

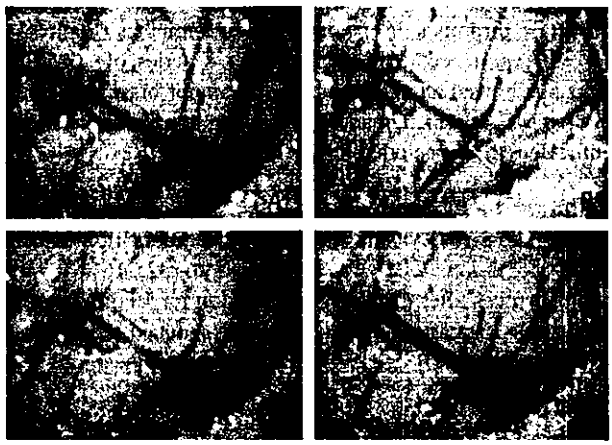

Figure 5. A, Distal MCA before photothrombotic occlusion. Bar=1 mm. B, Occluded distal MCA (bracket). C, At 1 minute of YAG laser irradiation, the occluded distal MCA was partially reperfused. D, Reperfused distal MCA.

mic brain.[21-23] Therefore, this photochemically induced MCA occlusion model would facilitate greater understanding of the pathophysiology of thrombotic stroke.

The mechanisms of ultraviolet laser induced–vascular dilatation are attributed to rapidly expanding vapor bubble formed intraluminally in smooth muscle at high energy density[24] or photolytic release of nitric oxide at low energy density.[25] Ultraviolet laser–induced dilatation facilitated formation of the microscopic intrathrombus channels that lead to recanalization of a platelet-occluded artery. The early reperfusion apparently confers advantages on ischemic brain tissue at risk, yet the return of blood flow in the postischemic brain has a negative side. For instance, a late secondary drop in the apparent diffusion constant was observed in some ischemic tissues of both rats[26,27] and humans[28] that appeared initially to be salvaged by reperfusion therapy. Reperfusion injury may be possibly related to this secondary deterioration. Hence, the therapeutic time window for reperfusion may be prolonged when combined with treatments that counteract reperfusion injury. Antagonists of various factors such as excitotoxicity, spectrin breakdown, free radicals, or apoptosis may be desirable in combination with reperfusion therapy.

In conclusion, we have characterized our SHR stroke model utilizing a 2-laser system, one to induce cerebral arterial occlusion and the other to facilitate its elimination. This model fulfills the standard of an acceptable focal ischemia model (ie, highly reproducible infarct volume) with a less invasive surgical procedure.

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Brain ischemia augments exo-focal transgene expression of adenovirus-mediated gene transfer to ependyma in hypertensive rats

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Abstract

The ependyma is one of the feasible targets for gene transfer to the brain. Using two different replication-deficient recombinant adenoviral vectors, AdCMVβGal or AdRSVIL10, we examined effects of cortical brain ischemia on transgene expression in the ependyma after administration of the vector into the lateral ventricle of spontaneously hypertensive rats (SHR). Expression of the reporter gene lacZ at the lateral ventricle was detected by histochemistry for semiquantitative scoring or by biochemical assay for quantitative analysis. Ependymal cells in the ventricles expressed the transgene as early as 6 h after gene transfer in both sham treatment and ischemia treatment. In the sham treatment, the expression peaked at 12 h and slowly decreased toward day 4 and day 7. However, transgene expressions in the ischemic brain on day 4 and day 7 were significantly higher than sham treatment. In the biochemical assay, β-galactosidase activity detected on day 4 at the periventricular area of the ischemic group (37 ± 9 mU/mg protein) was significantly greater than that of the sham group (12 ± 4, $P < 0.01$). In the enzyme-linked immunosorbent assay for gene transfer of interleukin-10 (IL-10), IL-10 in the cerebrospinal fluid (CSF) of the ischemic group (11,633 ± 4322 pg/ml) was significantly greater than that in the sham group (2460 ± 1486, $P < 0.05$) on day 5. These results suggest that transgene expression in the exo-focal remote area of ependyma is augmented by cortical ischemia, and the ependyma may be a promising target of gene transfer of brain ischemia.
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Keywords: Gene therapy; Adenovirus; Cerebral ischemia; Ependyma; IL-10

Introduction

Gene transfer is an attractive intervention for studies of basic mechanisms of neurobiology, and potentially for therapy of cerebrovascular diseases (Heistad and Faraci, 1996; Verma and Somia, 1997). Although several studies have shown usefulness of gene transfer to the brain to protect against ischemic damage, vectors have already introduced in the brain before induction of brain ischemia in those studies (Betz et al., 1995; Lawrence et al., 1996; Linnik et al., 1995; Yenari et al., 1998). To rationalize gene therapy for brain ischemia, it is important to show efficacy of gene transfer even when vectors are administered after induction of brain ischemia. We have shown recently that a reporter gene is successfully transfected to the ischemic penumbra after brain ischemia (Ooboshi et al., 2001).

Administration of the adenovirus into the lateral ventricle has produced extensive expression in the ependymal cells (Abe et al., 1998; Bajocchi et al., 1993; Ooboshi et al., 1995), and preinjection of adenovirus carrying interleukin-1 (IL-1) receptor antagonist into the cerebral ventricle reduced infarct volume (Betz et al., 1995; Yang et al., 1997). Therefore, the ependyma may be a good target for gene therapy of stroke. However, there are few reports that examine effects of brain ischemia on transgene expression in the ependyma after brain ischemia (Abe et al., 1998).

In this experiment, focal brain ischemia in the cortex was produced by photochemical occlusion of the distal middle cerebral artery (MCA) of spontaneously hypertensive rats (SHR), which provided a reproducible cortical infarction with a simple procedure (Yao et al., 1996). We delivered an adenoviral vector into the lateral ventricle after induction of cortical brain ischemia or sham operation, and examined

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effect of the brain ischemia on time course and distribution of transgene expression in the exo-focal area of ependyma and the cerebrospinal fluid (CSF).

Materials and methods

Adenoviral vectors

We used two different replication-deficient recombinant adenoviral vectors, AdCMVβGal for Experiment 1 and Experiment 2, and AdRSVIL10 for Experiment 3. The method for construction of vectors was described previously (Davidson et al., 1994; Rich et al., 1993). Briefly, the DNA constructs comprised of a full-length copy of the adenovirus genome of approximately 36 kb, from which the early region 1 gene (E1) was replaced by a cytomegalovirus (CMV) promoter or Rous sarcoma virus (RSV) promoter and a cDNA for *Escherichia coli* β-galactosidase gene with a simian virus 40 nuclear localization signal or human IL-10 gene (AdCMVβGal, AdRSVIL10). Recombinant viruses were grown in human embryonic kidney (HEK) 293 cells that complemented the E1 early viral promoters (Davidson et al., 1994), and were triple plaque purified to assure that viral suspensions were free of wild-type viruses. Viral titer was determined by plaque assay on HEK 293 cells. After purification, the virus was suspended in phosphate-buffered saline (PBS) with 3% sucrose, and was kept at −80°C until used.

Experiment 1

Animals and surgical procedure

All animal procedures were approved by the Animal Care and Use Review Committee at the Kyushu University (12-053-0). Forty-eight female SHR, aged 5–7 months and weighed 195–240 g, were used for Experiment 1. 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/30% oxygen. The right femoral artery and vein were cannulated using PE-50 tubing. The rats were endotracheally intubated with PE-240 tubing. Pancuronium bromide (an initial dose of 0.3 mg followed by 0.1 mg every 30 min) was intravenously injected, and the rats were mechanically ventilated. Mean arterial pressure was continuously monitored. Physiological variables were determined before and 1 h after the distal MCA occlusion. Rectal and head temperature was maintained at 37°C and 36°C, respectively, by means of a warming lamp and a heat pad.

The rat was 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 temporal muscle was separated and, under an operating microscope, a burr hole 3 mm in diameter was made 1 mm posterior to the anterior junction of the zygoma and squamosal bone, revealing the distal segment of MCA above the rhinal fissure. The dura was left intact.

CBF before and during ischemia at the parietal cortex was measured by laser Doppler flowmetry. A burr hole, 2 mm in diameter, was made in the parietal cortex at 4 mm lateral and 1.5 mm posterior to the bregma in the ipsilateral to ischemic side. The resting CBF value was regarded as baseline and changes after induction of brain ischemia were expressed as percentages of the resting value.

Brain ischemia

Brain ischemia was produced by photochemical occlusion of the distal MCA of SHR as described previously (Yao et al., 1996). A krypton laser operating at 568 nm (Innova 301, Coherent Inc.) 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) and positioned with a mirror onto the distal MCA. The photosensitizing dye, rose bengal (15 mg/ml in 0.9% saline; Wako Pure Chemical), was administered intravenously to a body dose of 20 mg/kg over 90 s simultaneously with 4 min of laser irradiation.

Injection of adenoviral vector

For the injection of adenoviral vectors into the lateral ventricle contralateral to the ischemic side, a small burr hole was made in the parietal region (1.0 mm posterior and 1.0 mm lateral to the bregma) with a dental drill. Ninety minutes after induction of ischemia, the recombinant virus was injected into the lateral ventricle. A 27-G needle on a Hamilton syringe was stereotaxically inserted into the left lateral ventricle (3.5 mm in depth), and 20 μl of viral suspension (3×10^{10} plaque forming units/ml) of AdCMVβGal was injected over 10 min. In the sham operation group, the same surgical procedure, including cannulation and craniotomy, and injection of viral suspensions were performed without occlusion of the MCA. Two hours after the distal MCA occlusion or sham operation, the head wound was closed and the catheters were removed. The rats were carefully weaned from the respirator and returned to the home cage after regaining the ability to breathe independently. After the injection of vectors, the rats in the ischemia group were housed for 6 h ($n = 3$), 12 h ($n = 4$), 1 day ($n = 4$), 4 days ($n = 5$), or 7 days ($n = 5$), and the rats in sham treatment group were housed for 6 h ($n = 3$), 12 h ($n = 4$), 1 day ($n = 4$), 4 days ($n = 9$), or 7 days ($n = 7$).

Histochemical analysis of gene expression

After the designated survival periods, the rats were anesthetized with amobarbital (100 mg/kg i.p.) and perfused transcardially with 2% paraformaldehyde and 0.2% glutar-

aldehyde in PBS. The brain was removed and washed thoroughly with PBS. The brain was cut into coronal sections at intervals of 2 mm and incubated in 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal, Wako Pure Chemical) staining solution for 3 h at room temperature, rinsed in PBS, and post-fixed with 4% formaldehyde. Incubation with X-Gal was limited to 3 h to prevent staining of endogenous β-galactosidase, which may be seen in the cytosol after longer (>4 h) periods of incubation (Lal et al., 1994). Efficacy of transgene expression to the brain was assessed at 6 h, 12 h and 1 day, 4 days and 7 days after injection of AdCMVβGal. Four slices that contained lateral ventricles were examined for positive staining of β-galactosidase (blue staining) in the macroscopic view. Expression of β-galactosidase in the lateral ventricle on the ischemic side was analyzed semiquantitatively, and estimated with a four-point scale: 0 (no stain), 1 (modest: approximately 1–25% area of stained blue in the ventricular wall), 2 (moderate: 26–75% area of stained blue in the ventricular wall) or 3 (marked: >75% area of stained blue in the ventricular wall) as described previously (Muhonen et al., 1997; Ooboshi et al., 2001). The scores of four slices were averaged and used for expression score. The fixed tissue was then processed for paraffin embedding, and sections (5 μm thick) were cut from the block with microtomes, placed on slides and counter-stained with nuclear fast red.

Experiment 2

Biochemical assay for transgene

Eight female SHR, aged 5–6 months, were quantitatively analyzed for transgene expression in Experiment 2. In this experiment, procedures for sham operation (n = 4) and MCA occlusion (n = 4) were similar to Experiment 1. Rats that survived for 4 days were used for biochemical assay of transgene as reported previously (Ooboshi et al., 2001; Takada et al., 2003). Briefly, rats were perfused with ice-cold PBS, and the brain was cut into coronal slices at intervals of 2 mm. From two coronal slices (the injected and the next posterior), several tissue sections were dissected out as follows: periventricular areas (2 mm long and 1 mm wide along the outer lateral wall) from both hemispheres, cortical areas (2-mm long cube at the parietal cortex) from both hemispheres. The brain sample was minced with a scalpel blade and lysed with 50 μl of lysis buffer containing 0.2% Triton X-100 and 100 mM potassium phosphate, pH 7.8. The suspension of brain tissue was centrifuged at 10,000 × g for 10 min and the supernatant was assayed for β-galactosidase activity using the Aurola GAL-XE assay kit (Wako Pure Chemical). Light emission was measured with a luminometer, MiniLumat LB 9506 (Berthold, Osaka, Japan), and calibrated with a standard curve generated using purified *E. coli* β-galactosidase (Boehringer Mannheim). Protein concentrations were determined using a Protein Assay CBB kit (Nacarai Tesque, Tokyo, Japan), and used for the correction of β-galactosidase activity as mU β-galactosidase/mg protein. Background values for chemiluminescence were measured in brain tissue from the non-treated rats (Control; n = 5). Assay was duplicated in each sample, and the averaged values were used. The normalized β-galactosidase activity of the tissue from the two slices was averaged.

Experiment 3

ELISA for human IL-10

Nine male SHR, aged 8–12 months, were quantitatively analyzed for transgene expression of human IL-10 in Experiment 3. In this experiment, procedures for sham operation (n = 5) and MCA occlusion (n = 4) were similar to Experiment 1, except for the volume and the type of adenoviral vector. Thirty microliters of AdRSVIL10 (3×10^{10} plaque forming units/ml) was stereotaxically injected into the left lateral ventricle over 10 min. Rats that survived for 5 days were anesthetized with pentobarbital (65 mg/kg i.p.) and CSF was withdrawn (Ooboshi et al., 1995, 1997b). Human IL-10 in the CSF was measured by sandwich enzyme-linked immunosorbent assay (ELISA). The ELISA kit (Biosource International, Camarillo, CA, USA) with the monoclonal antibody was used according to the manufacturer's direction (Osugi et al., 1997). The antibody does not cross-react with the rat IL-10.

Statistical analysis

Data are presented as mean ± SEM. Differences in physiological variables and human IL-10 between groups were analyzed with unpaired t test. Differences in β-galactosidase activity were analyzed with ANOVA followed by Bonferroni's post hoc t test. Differences in grading scores of transgene expression among the different regions were

Table 1
Physiological variables

	Sham (N = 27)	Ischemia (N = 21)
Body weight (g)	215 ± 2	218 ± 3
Age (month)	5.8 ± 0.1	5.8 ± 0.1
Head temperature (°C)	36.0 ± 0	36.0 ± 0
Rectal temperature (°C)	37.0 ± 0	37.0 ± 0
MAP (mm Hg)	170 ± 2	169 ± 2
Ht (%)	41.6 ± 0.2	41.5 ± 0.3
pH	7.43 ± 0.01	7.42 ± 0.01
$PaCO_2$ (mm Hg)	38 ± 1	39 ± 1
PaO_2 (mm Hg)	122 ± 4	121 ± 3
BS (mg/dl)	146 ± 3	149 ± 3

MAP, mean arterial blood pressure; Ht, hematocrit; BS, blood sugar. Values are mean ± SEM.

analyzed by nonparametric Mann–Whitney U test. $P < 0.05$ was regarded as statistically significant.

Results

Physiological variables

Physiological variables in Experiment 1 are shown in Table 1. There were no significant differences in physiological variables between the sham and ischemia groups. Arterial pressure significantly ($P < 0.05$) increased after induction of ischemia (174 ± 3 mm Hg vs. 169 ± 2). Blood flow to the cortex ipsilateral to the occlusion side began to decrease within 10 min after focal ischemia and lasted for more than 60 min. The reduction was −72 ± 2% at 10 min and −69 ± 1% at 60 min.

Transgene expression

Expression of the reporter gene was consistently detected at the periventricular areas since 6 h to 7 days after gene

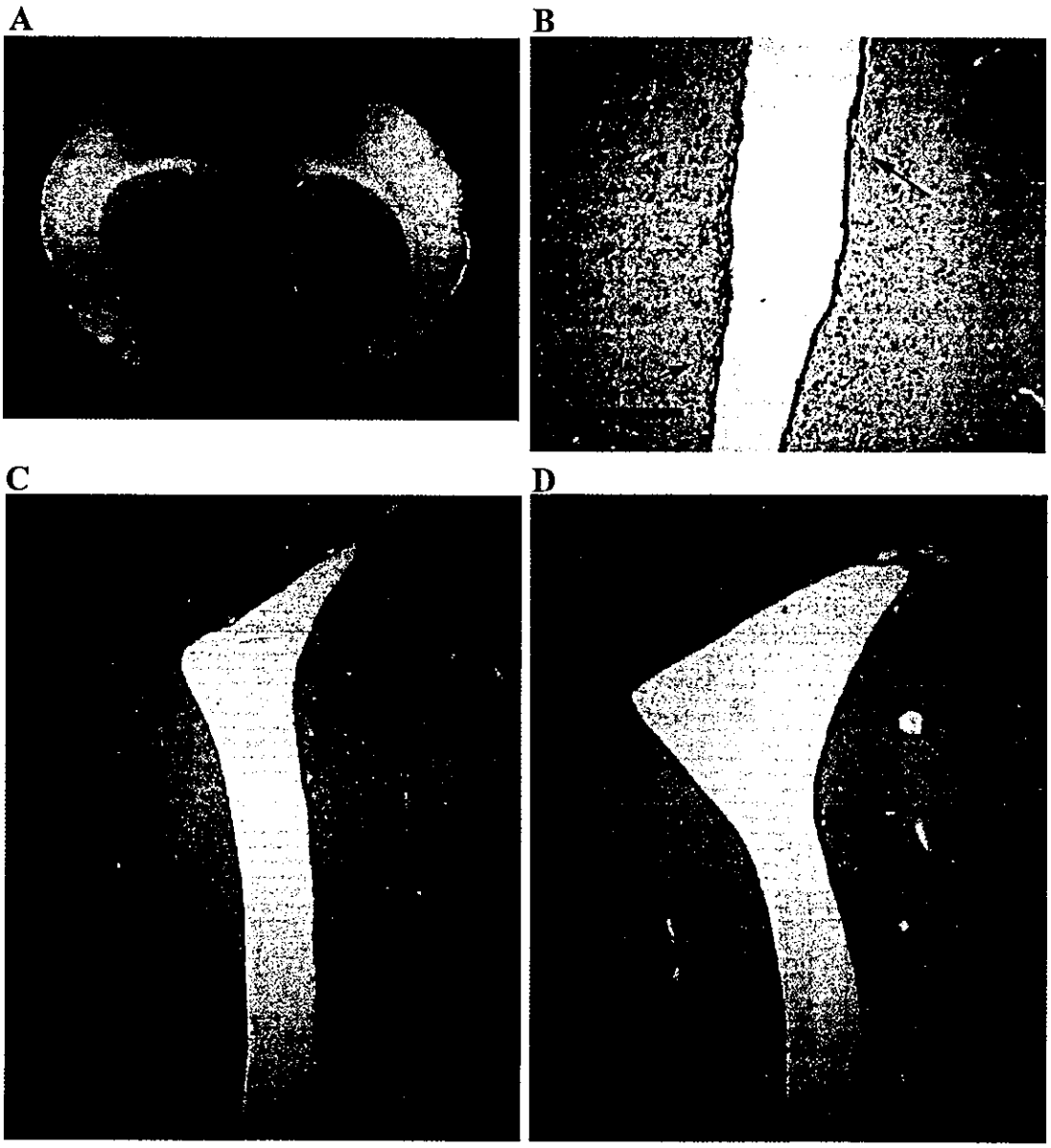

Fig. 1. Histochemical staining of rat brains after gene transfer with AdCMVβgal. Coronal section of the brain 4 days after the ischemic insult (A). Expressed transgene (β-galactosidase) at the lateral ventricles was stained with X-Gal. Brain ischemia was produced by photochemical occlusion of the distal middle cerebral artery. Microscopic view of the periventricular area in non-ischemic (B), and ischemic side (C) in the ischemic brain, and that in operated side in the sham-operated brain (D). Positive staining was observed mostly in ependymal cells and occasionally in subependymal cells (arrows). Slices were counterstained with nuclear fast red. Scale bar = 200 μm.

transfer in both sham and ischemia groups (Fig. 1A). X-Gal staining was not observed in the cortex. In the microscopic view, positive staining for β-galactosidase was detected mostly in the ependymal cell and a few positive cells were also observed in the subependymal cells (Fig. 1B). Four days after gene transfer, transgene expression at the periventricular area was observed more often in the ischemia group (Fig. 1C) than in the sham group (Fig. 1D).

Time course of semiquantitative analysis

Time course of semiquantitative analysis for transgene expression at the periventricular area on the ischemic side was demonstrated in Fig. 2. In the sham-treated rats, transgene expression was observed at the ependyma as early as 6 h after gene transfer, and the expression score was 0.42 ± 0.20 (mean ± SEM). The expression peaked at 12 h (1.50 ± 0.25) and slowly decreased to 0.28 ± 0.07 on day 4 and 0.07 ± 0.05 on day 7. In the ischemia group, transgene expression was found at 6 h (0.33 ± 0.20) and increased to 1.44 ± 0.19 at 12 h. However, expressions on day 4 (0.85 ± 0.29) and day 7 (0.45 ± 0.19) in the ischemia group were significantly higher than those in the sham group ($P < 0.05$).

Biochemical assay for quantitative analysis

Changes in physiological values and CBF of rats for quantitative analysis of transgene expression were similar with those of histochemical analysis (data not shown). Quantitative values of transgene in the periventricular areas were measured by biochemical assay and activity of β-galactosidase in the injected hemisphere tended to be greater in the ischemia group (354.6 ± 197.1 mU/mg protein) than the sham group (137.2 ± 53.5 mU/mg protein). In the ischemic (sham operated) hemisphere, amount of β-galactosidase at the periventricular area was significantly in-

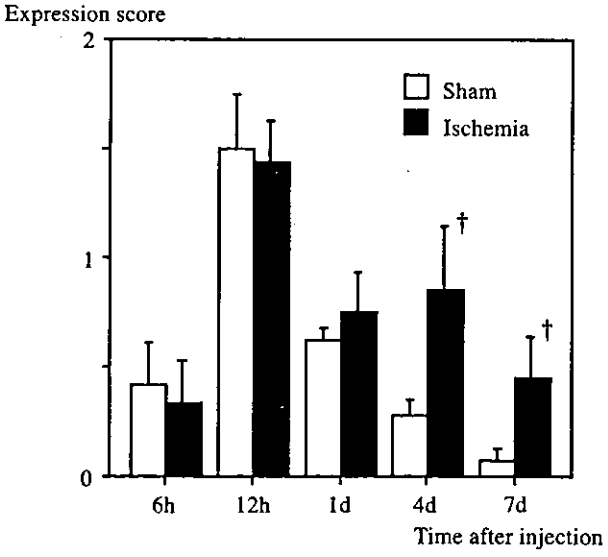

Fig. 2. Time course of transgene expression after gene transfer with AdCMVβgal in the ventricle on the ischemic side. Transgene expression was observed as early as 6 h after injection and peaked at 12 h, followed by gradual decreases. Transgene expression on day 4 and day 7 in the ischemic brain (Ischemia) was significantly greater than the sham-operated brain (Sham). † $P < 0.05$, Ischemia vs. Sham.

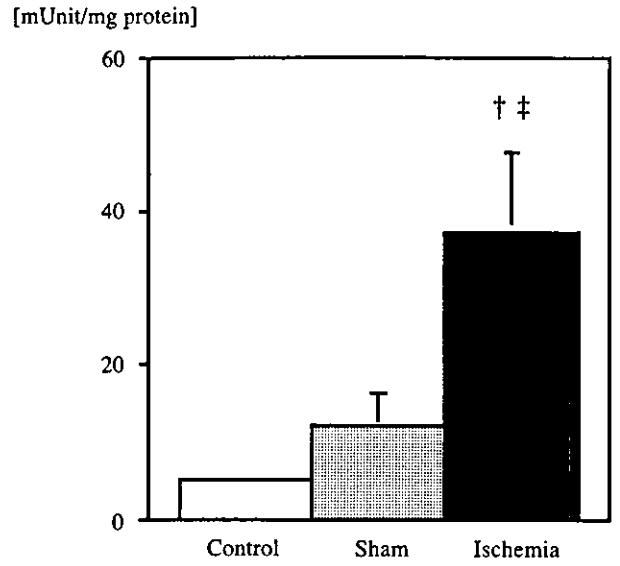

Fig. 3. Quantitative analysis of transgene expression after gene transfer with AdCMVβgal at the periventricular area on the ischemic side. Activity of β-galactosidase on the ischemic side was significantly increased in the ischemic brain (Ischemia) as compared with that in sham-operated brain (Sham) and non-operated brain (Control). Values are mean ± SEM. † $P < 0.01$, vs. Sham. ‡ $P < 0.005$, vs. Control.

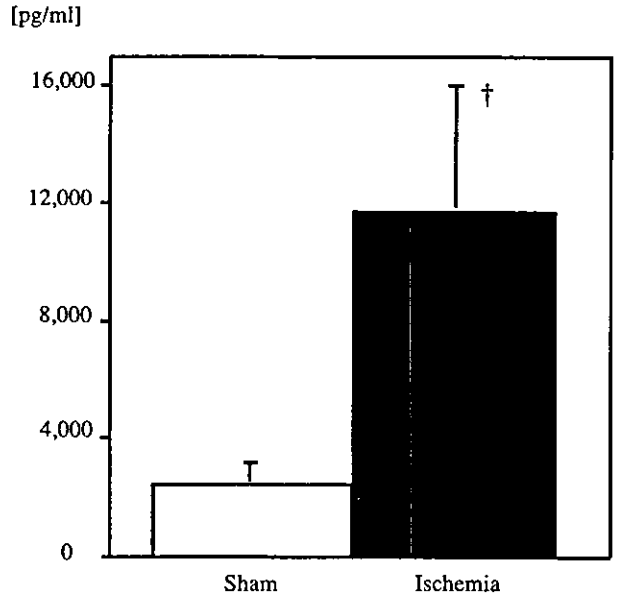

Fig. 4. Amount of human IL-10 in the CSF after gene transfer with AdRSVIL10. Human IL-10 on day 5 was significantly increased in the ischemic brain (Ischemia) as compared with that in sham-operated brain (Sham). Values are mean ± SEM. † $P < 0.05$, vs. Sham.

creased in the ischemic group (37.1 ± 10.7 mU/mg protein) as compared with that in the sham group (12.0 ± 4.2 mU/mg protein ; $P < 0.01$) and the control rats (5.0 ± 0.3 mU/mg protein ; $P < 0.005$) (Fig. 3). Activity of β-galactosidase in the parietal cortex was not different between the sham and ischemia groups in both hemispheres.

ELISA for human IL-10

Changes in physiological values and CBF of rats for quantitative analysis of transgene expression were similar with those of histochemical analysis (data not shown). Values of ELISA for IL-10 in the CSF are shown in Fig. 4. IL-10 on day 5 was significantly increased in the ischemic group (11,633 ± 4322 pg/ml) as compared with that in the sham group (2460 ± 1486 pg/ml; $P < 0.05$).

Discussion

In this experiment, we demonstrated that adenovirus-mediated gene transfer into the ventricle provided effective expression of transgene in the ependyma as early as 6 h after gene transfer in the presence or absence of preceding focal brain ischemia. Our major new finding was that transgene expression in the exo-focal area of ependyma was augmented by the remote cortical ischemia. The augmentation was provided in the different types of transgene driven by two promoter (CMV and RSV) systems.

Several studies reported that gene transfer was effective in reducing infarct size or attenuating neuronal damage (Betz et al., 1995; Lawrence et al., 1996; Linnik et al., 1995; Xu et al., 1997; Yang et al., 1997; Yenari et al., 1998). However, these studies were performed under the conditions that vectors for gene transfer were induced in the brain before ischemic insult. Although these studies provide the useful information regarding gene therapy, we need to evaluate efficacy of post-ischemic gene transfer for the clinical application. In the previous study, we showed efficiency of gene transfer to the ischemic penumbra even when vectors were administered after induction of brain ischemia (Ooboshi et al., 2001). However, efficiency of gene transfer to the ependyma, a potential target of gene therapy for stroke, remains to be elucidated.

In this study, we observed that transgene expression at the ependyma was detected as early as 6 h after gene transfer, and peaked at 12 h, followed by gradual decline. Abe et al. (1998) described effective transgene expression using an adenoviral vector with CMV promoter in the ventricular cells at 8 h to 7 days after transient global ischemia. Although they mentioned good expression in ischemic tissue, quantitative analysis was not performed. Yang et al. (1997) injected the adenoviral vector with RSV promoter and IL-1 receptor antagonist gene into the cerebral ventricle 5 days before focal brain ischemia, and observed transgene expression as early as 1 day after injection, followed by peak expression at 5–7 days. The peak of transgene expression was reported to be quicker and greater with CMV promoter than RSV promoter when administered to the cistern (Christenson et al., 1998). Therefore, gene transfer to the ependyma using CMV promoter may be a suitable approach for treatment of brain ischemia when overexpression of protective genes acts in the acute phase of ischemic cascade.

In our study, cortical ischemia augmented adenovirus-mediated transgene expression using two different promoters, CMV and RSV. CMV promoter has several repeated sequences, some of which are known to bind to nuclear factor kappa B (NFκB) and cyclic AMP response element-binding protein (CREB), resulting in enhancement of binding of RNA polymerase II (Boshart et al., 1985; Sambucetti et al., 1989). In fact we and others (Christenson et al., 1999; Clesham et al., 1996) have observed that gene transfer of the reporter gene with CMV promoter is augmented with phorbol 12-myristate 13-acetate or forskolin, which presumably stimulates the promoter through CREB binding. Thus, in the inflammatory conditions, including post-ischemic processes, transgene expression would be stimulated through NFκB binding when CMV promoter is used (Ooboshi et al., 1997a), and the augmentation of transgene in this study may be partly attributable to stimulation of the promoter.

Modulation factors for RSV promoter are less well characterized. In contrast to the CMV, RSV promoter is irresponsive to the increased cyclic AMP levels (Mellon et al., 1989). Recently, YY1, a transcriptional activator, has been reported to augment the transcription driven by RSV promoter (Bhalla et al., 2001). Because inflammatory stimuli were suggested to induce YY1 (Gordon et al., 2003), the ischemic injury may activate the RSV promoter. Thus, transgene expression in the ependyma could be augmented under these commonly used promoter systems.

In this experiment, focal brain ischemia in the cortex was produced by photochemical occlusion of the distal MCA, and the ependyma was not exposed to ischemia directly (Yao et al., 1996). There are some reports that revealed direct effects of focal or global brain ischemia on gene transfer (Abe et al., 1998; Ooboshi et al., 2001). However, the indirect or remote effects of ischemia on gene transfer have not been reported. In our study, focal brain ischemia in the cortex augmented transgene expression in the exo-focal remote area of ependyma. Because activity of β-galactosidase in the ischemic cortex was not different from sham treatment, the augmentation in the ependyma was not associated in the activation of endogenous β-galactosidase by ischemia. One possible cause of such indirect augmentation is the induction of stimulatory factors, such as cytokines (Arvin et al., 1996; Stoll et al., 1998). Inductions of TNF-α and IL-1β occur after focal brain ischemia at ischemic lesions and at noninfarcted ipsilateral cortex as well, but not in contralateral hemisphere (Jander et al., 2000). In our experiment, the augmentation of transgene expression as the remote effect was much greater at the ipsilateral hemisphere than the contralateral to ischemia. Taken together with the

time course study, the induced cytokines by focal brain ischemia may augment transgene expression in the later stage of brain ischemia.

Finally, we occasionally observed transgene expression in the subependymal cells. Recent reports have revealed that neural stem cells locate in the subependymal layer, and some studies suggest the possibility that the ependymal cells are neurostem cells (Goldman et al., 1996; Johansson et al., 1999). In addition, focal brain ischemia has been reported to induce the neurogenesis in the subventricular zone in rats (Jin et al., 2001). Therefore, a portion of the augmented transgene expression in the ventricular wall might represent the stimulated neurogenesis. The future modulation of neurogenesis by the gene transfer to the ependymal and subependymal cells may lead to therapeutic strategy for efficient functional recovery after stroke.

In conclusion, focal brain ischemia in the cortex augmented adenovirus-mediated transgene expression in the exo-focal area of ependyma. Gene transfer to the ependyma may be a promising approach for treatment of brain ischemia.

Acknowledgments

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脳梗塞急性期患者の実態

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要　旨

脳卒中データベースにより集積された急性期脳梗塞患者について，脳梗塞病型別頻度，国際比較，時代的推移，危険因子を中心に，従来の他の調査とも比較しながら簡述した．動脈硬化を基盤としたラクナ梗塞，アテローム血栓性脳梗塞には高血圧，糖尿病，高脂血症が多く合併し，心原性脳塞栓症では心房細動の合併が多かった．近年，アテローム血栓性脳梗塞の頻度が増加しているが，その原因として本調査参加施設の特徴と我が国における食生活の欧米化などが考えられた．

はじめに

従来より日本人には脳卒中が多く，欧米白人には虚血性心疾患のリスクが高いことが特徴とされてきた．過去 30 年間で，我が国の脈管病も少しずつ様変わりを呈し，今では欧米諸国と肩を並べるまでになった[1]．危険因子の頻度や諸因子のかかわりの度合いも時代とともに移り変わりつつあり[2]，その原因の1つとして我が国の生活習慣や環境の欧米化が挙げられるであろう．しかし，脳卒中は相変わらず代表的国民病として君臨しており，患者の絶対数は決して減っていないのが実状である．

本稿では，我が国の急性期脳卒中患者データベース（8,000 例）から発症後1週間以内に入院した脳梗塞 4,979 例を抽出し，脳梗塞における病型別・年代別・性別頻度と危険因子の実態について解析した成績を中心に述べる．

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キーワード：脳梗塞，臨床病型，危険因子

脳梗塞病型分布の国際比較，時代的推移

脳梗塞は発症機序や責任血管の大きさによって，ラクナ梗塞，アテローム血栓性脳梗塞，心原性脳塞栓症の3つの臨床病型に分けられる．全国の急性期脳卒中救急診療の拠点病院を中心に，1999 から 2001 年度までの3年間に集積した 4,979 例の急性期脳梗塞患者の病型別内訳では，ラクナ梗塞（1,528 例）に対し血栓性梗塞と血栓性塞栓を合わせたアテローム血栓性脳梗塞（1,535 例）がほぼ同数で，結果的に脳梗塞の各臨床病型はいずれも 1/3 ずつの分布を示した（図1）．一過性脳虚血発作は脳梗塞ではないので図には含まれないが，478 例（男性 307 例，女性 171 例）に存在した．

脳梗塞病型分布を国際的に比較すると，欧米ではアテローム血栓性脳梗塞と心原性脳塞栓症の頻度がラクナ梗塞より高い（表1）[3~9]．

図1 脳梗塞病型分布

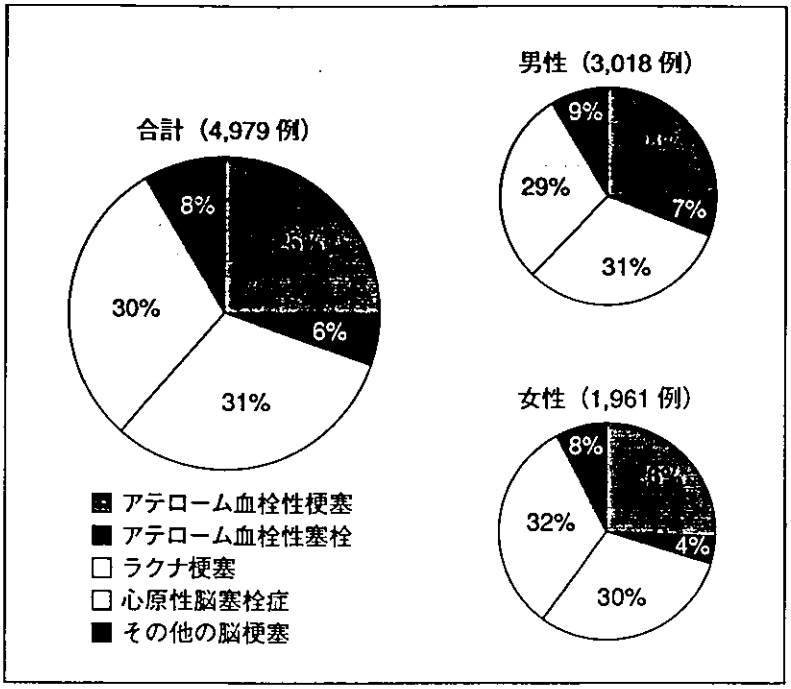


表1 脳梗塞病型分布の国際比較

調査	期間	人数	脳梗塞			
			アテローム血栓性脳梗塞	ラクナ梗塞	心原性脳塞栓症	その他の脳梗塞
本調査	1999～2001	4,979	31%*	31%	30%	8%
久山町	1961～1993	298	21%	56%	19%	4%
国立循環器病センター	1978～1991	1,216	22%	42%	27%	9%
J-MUSIC	1999～2000	15,831	33%	39%	22%	6%
米国1	1972～	583	40%	23%	37%	0%
米国2	1980～1981	708	24%	14%	28%	33%
米国3	1985～1989	290	26%	25%	46%	4%
スイス	1982～	778	55%	19%	26%	0%

*：アテローム血栓性梗塞 25%，アテローム血栓性塞栓 6％

J-MUSIC：Japan Multicenter Stroke Investigator's Collaboration,

米国1：Harvard Cooperative Stroke Registry，米国2：Pilot Stroke Data Bank,

米国3：Rochester Epidemiology Project Medical Records Linkage System，スイス：Lausanne Stroke Registry

一方，我が国において，1961 年から行われた久山町研究と国立循環器病センターの入院患者統計では，アテローム血栓性脳梗塞が少なくラクナ梗塞が多い．それに対し，ごく最近の本調査や全国 156 施設で行われた山口班々会議調査である J-MUSIC では，アテローム血栓性脳梗塞が増加している．さらに，時代的推移について久山町3集団の脳梗塞発症例（65 歳以上男性）を病型別に分けて比較すると，ラクナ梗塞が時代とともに減少し，相対的にアテローム血栓性脳梗塞，心原性脳塞栓症の頻度が増加していることが分かる

図2　脳梗塞発症例の病型分布の時代的推移（久山町3集団，65歳以上男性，追跡各8年）

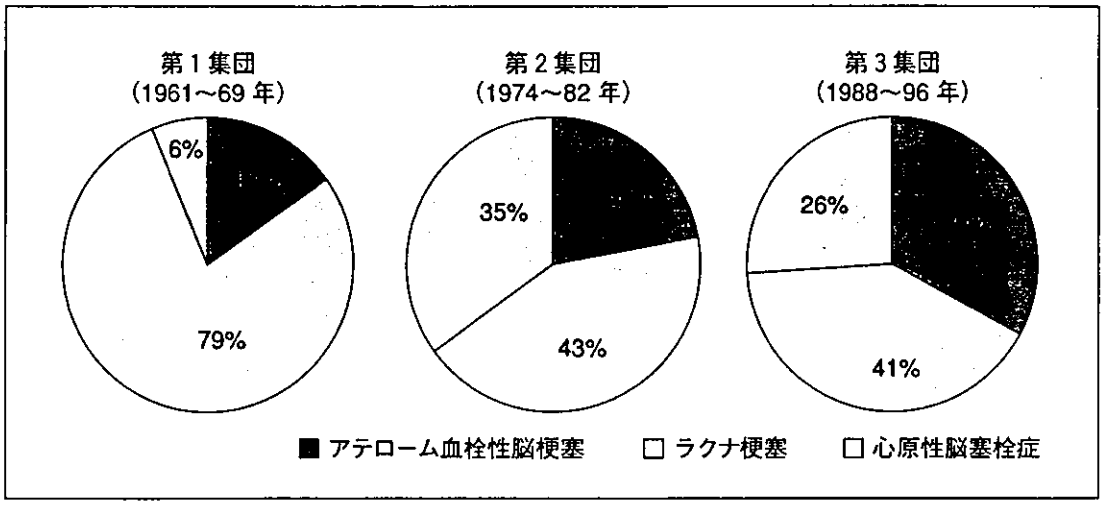
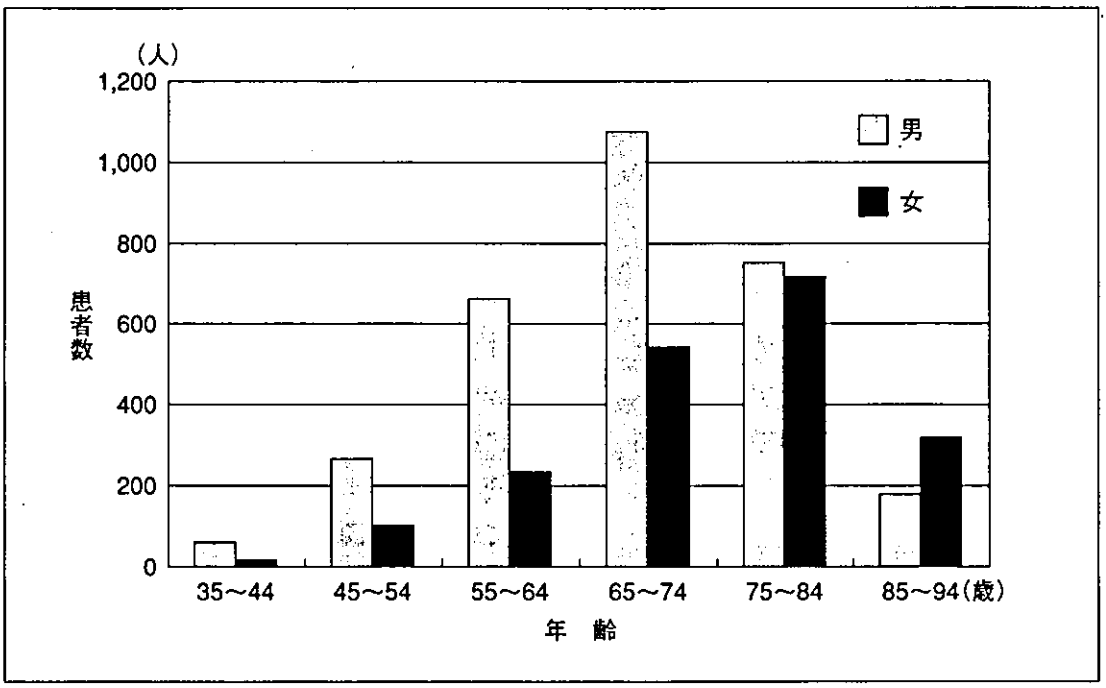


図3　年齢階級別にみた脳梗塞発症患者数



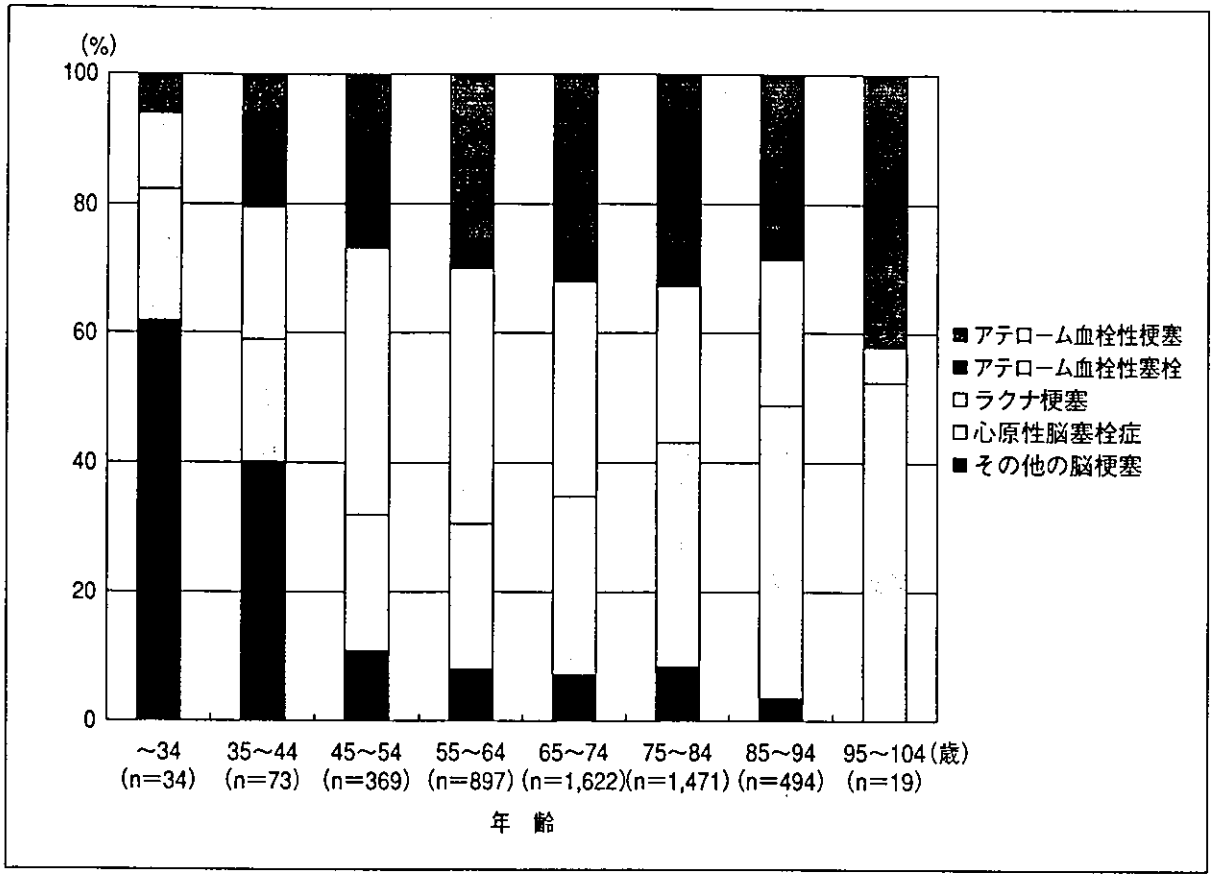
(図2)[10]．脳梗塞の臨床病型を国際的に比較し，時代的推移を検討することで，我が国の脳梗塞が欧米化しつつあることが示唆された．

年齢階級別にみた脳梗塞の発症患者数と臨床病型分布

本調査における年齢階級別にみた脳梗塞発症患者数のグラフを見ると，男性は加齢に伴って増加し65～74歳に頂値があるのに対し，女性では75～84歳に頂値があった（図3）．Framingham研究では，1950年に5,184名（30～62歳）の住民を対象に40年間の追跡調査を行い，その後のアテローム血栓性脳梗塞の発症患者数について検討しているが，その結果は本調査とほぼ同様であった[11]．

年齢階級別にみた脳梗塞の臨床病型では，加齢に伴って心原性脳塞栓症が増加するのに

図4 年齢階級別にみた脳梗塞の臨床病型



対し，ラクナ梗塞は反対に減少していた．血栓性梗塞と血栓性塞栓を合わせたアテローム血栓性脳梗塞は，加齢に伴って緩やかに増加していた（図4）．

病型別・年代別・男女別の危険因子

脳卒中の危険因子は，管理可能な因子と管理不能な因子とに大別される．前者には高血圧，糖尿病，高脂血症，心房細動，血液異常などの疾病，ならびに肥満，喫煙，飲酒，ストレス，食事などのライフスタイル上の悪習慣が含まれ，後者には年齢，性，遺伝，人種，季節といった因子が含まれる．本調査に基づいて，脳梗塞の臨床病型別にみた危険因子の合併頻度を表2に，若年群（49歳以下），壮年群（50歳以上69歳以下），老年群（70歳以上）の年代別にみた危険因子の合併頻度を表3に，さらに男女別の危険因子の合併頻度を表4に示した．

家族歴[12]についてみると，脳梗塞の臨床病型別に検討した報告[13]では，アテローム血栓性脳梗塞とラクナ梗塞に脳卒中の家族歴が寄与していた．本調査では，アテローム血栓性脳梗塞に関連が強かった．年代別では壮年群に比べ老年群で少なく，性別での有意な差は認めなかった．

脳卒中の既往歴については，アテローム血栓性脳梗塞が心原性脳塞栓症とその他の脳梗塞に比べて多かった．さらに，脳卒中の既往歴は加齢に伴って増加傾向を認め，女性に比べ男性に多く認められた．

高血圧は脳卒中の最大の危険因子であり，血圧が高くなるほど発症率は上昇する．高血圧治療により，脳卒中の死亡率や発症率を約40%近くも減少することが以前から示されている[14]．本調査における高血圧は，アテ

表2　脳梗塞臨床病型別の危険因子頻度の比較

	アテローム血栓性脳梗塞 (1,535例)	ラクナ梗塞 (1,528例)	心原性脳塞栓症 (1,496例)	その他の脳梗塞 (420例)
家族歴	237 (15.4%)†	213 (13.8%)	183 (12.2%)	63 (15.0%)
既往歴	446 (29.1%)†	406 (26.6%)	375 (25.1%)	94 (22.4%)
高血圧	926 (60.3%)*	984 (64.4%)*	715 (47.8%)	184 (43.8%)
糖尿病	443 (28.9%)*	436 (28.5%)*	240 (16.0%)	69 (16.4%)
高脂血症	366 (23.8%)*	399 (26.1%)*	187 (12.5%)	75 (17.9%)‡
心房細動	93 (6.1%)*	67 (4.4%)*	974 (65.1%)	18 (4.3%)*
喫煙歴	502 (32.7%)*	539 (35.3%)*	376 (25.1%)	160 (38.1%)*
飲酒歴	380 (24.8%)	375 (24.5%)	316 (21.1%)	114 (27.1%)‡

†：P<0.05 対 心原性脳塞栓症，χ^2 検定，*：P<0.0001 対 心原性脳塞栓症，χ^2 検定，
‡：P<0.01 対 心原性脳塞栓症，χ^2 検定

表3　年代別の危険因子頻度の比較

	若年群 49歳以下 (218例)	壮年群 50～69歳 (1,894例)	老年群 70歳以上 (2,867例)
家族歴	33 (15.1%)	295 (15.6%)	366 (12.8%)†
既往歴	21 (9.6%)*	475 (25.1%)	815 (28.4%)‡
高血圧	68 (31.2%)*	1,125 (59.4%)	1,369 (47.8%)*
糖尿病	31 (14.2%)*	575 (30.4%)	582 (20.3%)*
高脂血症	43 (19.7%)	483 (25.5%)	501 (17.5%)*
心房細動	13 (6.0%)*	320 (16.9%)	819 (28.6%)*
喫煙歴	103 (47.2%)	831 (43.9%)	633 (22.1%)*
飲酒歴	78 (35.8%)	640 (33.8%)	467 (16.3%)*

†：P<0.001 対 壮年群，χ^2 検定，*：P<0.0001 対 壮年群，χ^2 検定，
‡：P<0.05 対 壮年群，χ^2 検定

表4　男女別の危険因子頻度の比較

	男性 (3,018例)	女性 (1,961例)	P
家族歴	432 (14.3%)	262 (13.4%)	N.S.
既往歴*	854 (28.3%)	467 (23.8%)	0.005
高血圧	1,664 (55.1%)	1,145 (58.4%)	<0.05
糖尿病*	757 (25.1%)	431 (22.0%)	<0.05
高脂血症	598 (19.8%)	429 (21.9%)	N.S.
心房細動	677 (22.4%)	475 (24.2%)	N.S.
喫煙歴*	1,431 (47.4%)	146 (7.4%)	<0.0001
飲酒歴*	1,109 (36.7%)	76 (3.9%)	<0.0001

*：男性に高頻度な危険因子

ローム血栓性脳梗塞とラクナ梗塞に多く，50歳以上の壮年・老年群に多く，そして女性に多く認められた．

糖尿病は脳梗塞の中でもアテローム血栓性脳梗塞とラクナ梗塞に寄与している[13]．本調査でも糖尿病はアテローム血栓性脳梗塞とラクナ梗塞に多く，50歳以上の壮年・老年群での頻度が高く，男性に多く認められた．

高脂血症は脳梗塞の危険因子として挙げられ[15][16]，中でもアテローム血栓性脳梗塞に関連する危険因子と考えられている[16]．本調査ではアテローム血栓性脳梗塞とラクナ梗塞の双方に多く，また壮年群に多く認められた．

心房細動は心原性脳塞栓症で圧倒的に合併頻度が高く，年齢に伴って合併頻度が増加し，加齢の関与が示唆された[17]．

喫煙歴はラクナ梗塞の危険因子として報告されているが[3]，本調査ではその他の脳梗塞に最も多く，アテローム血栓性脳梗塞とラクナ梗塞でも多かった．若年であるほど，また喫煙量が多いほど，脳梗塞の相対危険が高くなるという報告もあり[18]，本調査における喫煙歴の年代別検討でも若年・壮年群に多かった．男女別では圧倒的に男性に多く認められた．

飲酒は，大量摂取で脳梗塞の危険因子となるが，少量ではむしろ危険率を低下させると言われている[19]．しかし，我が国では飲酒量と脳梗塞との相関関係は認めず[3]，本調査でも同様の結果であった（図には提示していない）．本調査での飲酒歴はその他の脳梗塞で多く，若年・壮年群に多く見られ，男性に多く認められた．

おわりに

我が国の急性期脳卒中患者データベースから，発症後1週間以内に入院した脳梗塞例を抽出し，病型別・年代別・性別頻度，危険因子の頻度などの実態を中心に検討し，その結果，脳梗塞病型の欧米化傾向が強く示唆された．欧米での成績を参考に，今後も各種生活習慣病に対する予防・治療が何よりも不可欠であると考えられた．今後もデータベースの構築の確立により，脳卒中の実態を把握し，積極的な予防対策に努めなければならない．

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Present Status of Acute Brain Infarction

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公　開　講　座

脳卒中の発症予防からボケないための対策について

日　時：平成13年12月8日　　場　所：福岡市役所15階講堂

座　長　九州大学名誉教授　　藤　島　正　敏

1.	脳卒中予防のための生活習慣病対策	井　林　雪　郎
2.	脳卒中の急性期の症状と治療法	岡　田　　靖
3.	脳卒中のリハビリテーション	竹之山　利　夫
4.	脳卒中の後遺症とボケ防止対策	長　尾　哲　彦
5.	脳卒中診療の流れ―急性期から慢性期へ	藤　井　健一郎
6.	脳卒中治療におけるかかりつけ医の役割	長　柄　　均
7.	脳卒中診療におけるリハビリテーション病院の役割	服　部　文　忠

座長のことば

九州大学名誉教授　藤　島　正　敏

私は2000年の3月に定年で九大を退官いたしまして，現在65歳となり，高齢者の仲間入りをしました。

本日ご出席の皆様方の約半数は65歳以上で，この中にはご自身が脳卒中を経験，あるいはご家族に脳卒中の方が少なからずいらっしゃるのではないかと存じます。実は，私の父親は80歳のときに第1回目の脳梗塞を発症，翌年再発し，5年後に再々発作のため86歳で亡くなりました。2回目の発作で失語症になりましたが，これが父親の生活の質（QOL）を著しく損ない，介護していました母親が音を上げてしまいました。脳卒中は患者さんご自身の苦しみとともに家族まで巻き込む非常に怖い，厄介な病気です。しかし，脳卒中は全く予防できないわけではありません。きょうは皆様方と一緒にこの脳卒中の予防を考えていきたいと思います。ここで日本の現状をスライドをごらんいただきながらお話しいたします。

1．久山町研究

私が勤めていました九州大学第二内科は，1961年（昭和36年）に福岡市に隣接する久山町で，町の全面的な協力のもとに脳卒中の疫学調査（久山町研究）を始めました。そもそもこの調査を始めることになった理由は，我が国は脳出血で亡くなる方が欧米諸国に比べて異常に多いことについて，アメリカの学者から日本のデータ（死亡統計）に疑問（脳卒中の診断が正しく行われていない？）が投げかけられたからです。しかし1950年代はそれを立証するエビデンス（根拠）がわが国にはなく，そこで疫学調査が始まったわけです。

この調査は先々代の故勝木司馬之助教授が始められ，次いで尾前照雄教授（現国立循環器病センター名誉総長）から私が16年間継ぎ，2001年から飯田三雄教授へとバトンタッチされ，すでに40年を過ぎました。

この久山町研究は40歳以上の住民が対象となり1961年から前向きに行われている調査で，これを「コホート（cohort）研究」と呼びます。九州大学のスタッフによって検診を行い，脳卒中が発症しますと往診をするという仕組みになっています。検診の受診率は80％以上，追跡率は99.8％で不幸にしてお亡くなりになった方は解剖させていただき，脳卒中のタイプ（病型）および死因を明らかにしました。剖検率は