tive inhibitor for MCP-1 and blocks the MCP-1/CCR2 signal pathway *in vivo* (Egashira et al., 2000; Egashira, 2003). This mutant MCP-1 and normal MCP-1 form a heterodimer, which binds to the MCP-1 receptor (CCR-2) and completely inhibits MCP-1–mediated monocyte chemotaxis *in vitro* (Rollins, 1996). In this study, we delivered the adenoviral vector encoding 7ND into the cerebral ventricle 90 minutes after focal brain ischemia, and examined whether anti–MCP-1 gene therapy would protect against focal brain ischemia.

MATERIALS AND METHODS

Adenoviral vectors

We used replication-deficient recombinant adenoviral vectors expressing *Escherichia coli* β-galactosidase (AdlacZ) or mutant MCP-1 (Ad7ND). An N-terminal deletion mutant of human MCP-1, called 7ND, lacks the amino-terminal amino acids 2 to 8. The 7ND was constructed by recombinant polymerase chain reaction using a wild-type human MCP-1 cDNA as the template and cloned into *Bam*HI (5′) and *Not*I (3′) sites of the pcDNA3 expression vector (Egashira et al., 2000, Ni et al., 2001). The DNA constructs of vectors composed of a full-length copy of the adenovirus genome of approximately 36 kb, from which the early region 1 gene (E1) was replaced by the CAG (cytomegalovirus enhancer, chicken β-actin enhancer-promoter and rabbit β-globin poly-A signal) promoter and a cDNA for lacZ or 7ND. Recombinant viruses were grown in human embryonic kidney (HEK) 293 cells that complemented the E1 early viral promoters, 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 further use.

Animals

All animal procedures were approved by the Animal Care and Use Review Committee at the Kyushu University (12-053-0). Twenty-eight male spontaneously hypertensive rats (SHR), aged 5 to 10 months and weighing 320 to 400 g, were used. Eighteen rats were semiquantitatively or quantitatively analyzed for transgene expression of β-galactosidase or 7ND, and 10 rats were used for the brain ischemia study.

Histochemical analysis of gene expression

Twelve male SHR were quantitatively analyzed for transgene expression of β-galactosidase. Briefly, rats were anesthetized with pentobarbital (65 mg/kg, intraperitoneal injection) and mounted on a stereotaxic head holder in the prone position. A 2-cm incision was made vertically midway. Rectal and head temperature was maintained at 37°C and 36°C, respectively, by means of a warming lamp and a heating pad. For the injection of adenoviral vectors into the left ventricle, a small burr hole was made in the parietal region (1.5 mm posterior and 1.0 mm lateral to the bregma) with a dental drill. A 27-G needle on a Hamilton syringe was stereotaxically inserted into the left lateral ventricle (4.5 mm deep), and 30 μL of AdlacZ (1.3×10^9 plaque forming units per milliliter, n = 12) was injected over 10 minutes. Efficacy of transgene expression to the brain was assessed 6 hours (n = 1), 12 hours (n = 2), 1 day (n = 3), 3 days (n = 2), 5 days (n = 3), and 7 days (n = 1) after injection of AdlacZ. After the designated survival periods, the rats were killed with an intraperitoneal injection of pentobarbital and perfused transcardially with 2% paraformaldehyde and 0.2% glutaraldehyde 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, Osaka, Japan) staining solution for 3 hours at room temperature, rinsed in PBS, and postfixed with 4% formaldehyde. Incubation with X-Gal was limited to 3 hours to prevent staining of endogenous β-galactosidase, which may be seen in the cytosol after longer periods (>4 hours) of incubation (Lal et al., 1994). Four slices that contained lateral ventricles in each rat were examined for positive staining of β-galactosidase (blue staining) in the macroscopic view. Expression of β-galactosidase in the left ventricle was analyzed semiquantitatively, and estimated with a four-point scale (0 = no stain, 1 = modest or approximately 1% to 25% area of stained blue in the ventricular wall, 2 = moderate or 26% to 75% area of stained blue in the ventricular wall, and 3 = marked or >75% area of stained blue in the ventricular wall) as described previously (Kumai et al., 2003; Ooboshi et al., 2001). The scores of four slices were averaged and used as expression score.

Measurement of 7ND

Six male SHR, weighing 370 to 400 g, were quantitatively analyzed for transgene expression of 7ND. In this experiment, procedures for operation were similar to those described for the previous experiment, except for the type of adenoviral vector. Thirty microliters of AdlacZ (1.3×10^9 plaque forming units per milliliter, n = 3) or Ad7ND (1.3×10^9 plaque forming units per milliliter, n = 3) was injected over 10 minutes. Six hours, 1 day, and 5 days after injection of vector, rats were anesthetized with pentobarbital (65 mg/kg, intraperitoneal injection) and the CSF was withdrawn (Ooboshi et al., 1995, 1997). The CSF concentration of 7ND released by transfected ependyma was measured by using sandwich enzyme-linked immunosorbent assay (ELISA) of human MCP-1. The ELISA kit (R&D Systems, Minneapolis, MN, U.S.A.) with the monoclonal antibody was used as reported previously according to the manufacturer's instructions (Kohara et al., 2002). The antibody does not cross-react with rat MCP-1.

Brain ischemia

Ten male SHR were used for the brain ischemia study. Briefly, rats were anesthetized with halothane (3% for induction; 1.5% during the surgical preparation, with a facemask; 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 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 minutes) was intravenously injected, and the rats were mechanically ventilated. Mean arterial pressure was continuously monitored. Physiologic variables were measured before and 1 hour after the distal middle cerebral artery (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 headholder 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. Cerebral blood flow (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 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., Santa Clara, CA, U.S.A.) 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, Irvine, CA, U.S.A.) 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 seconds simultaneously with 4 minutes of laser irradiation.

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 as the above experiments. Ninety minutes after induction of ischemia, 30 μL of viral suspension of AdlacZ (1.3×10^9 plaque forming units per milliliter, n = 5) or Ad7ND (1.3×10^9 plaque forming units per milliliter, n = 5) was injected into the lateral ventricle over 10 minutes. Two hours after the distal MCA occlusion, 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 were housed for 5 days.

Quantification of brain infarction and infiltration leukocyte

Five days after brain ischemia, rats were anesthetized with pentobarbital. The brain was removed, washed thoroughly with PBS, and cut into coronal sections at intervals of 2 mm followed by postfixation with 4% formaldehyde. The fixed tissue was then processed for paraffin embedding, and sections (5 μm thick) were cut from the block with microtomes for hematoxylin-eosin staining. Morphometric determination of infarct volume by the direct method has been described previously (Liu et al., 1989). The cross-sectional area of infarct was measured with NIH Image software (version 1.63) and infarct volume of each rat was calculated.

The number of polymorphonuclear and mononuclear leukocytes in blood vessels at the infarct area was determined in the coronal slice at the level of caudate-putamen by hematoxylin-eosin staining. The number of leukocytes was divided by infarct area, and was expressed as number per square centimeter.

Immunohistochemistry of macrophage

For immunohistochemistry, paraffin slices 5 μm thick at the caudate-putamen level were preincubated with 3% skim milk to decrease nonspecific binding. Sections were incubated overnight at 4°C with the mouse anti–rat macrophage/monocyte antibody (ED1, Serotec, Oxford, U.K.) diluted 1:1000, or nonimmune mouse IgG (Santa Cruz Biotechnology Inc., Santa Cruz, CA, U.S.A.) diluted 1:1000 as negative control. The slides were washed and incubated with biotinylated, affinity-purified rabbit anti-mouse IgG (Nichirei Corporation, Tokyo, Japan) as the secondary antibody. After avidin-biotin amplification, the slides were incubated with 3′,3′-diaminobenzidine. For quantification, the number of ED1-positive cells at the ischemic area was analyzed with NIH Image software (version 1.63), and was expressed as number per square millimeter. The slides were counterstained with hematoxylin for nuclear staining.

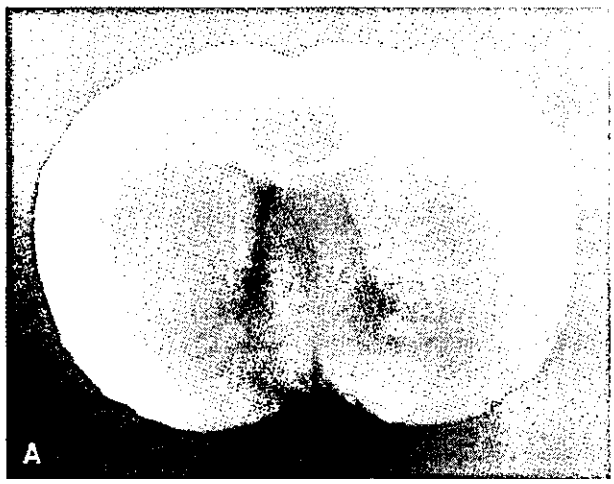
Statistical analysis

Data are presented as means and standard deviations. Differences in physiologic variables, infarct volume, and number of leukocytes and ED1-positive cells between groups were analyzed by unpaired *t*-test. Differences in amount of 7ND were analyzed with repeated measure one-way analysis of variance followed by Bonferroni post hoc *t*-test. $P < 0.05$ was regarded as statistically significant.

RESULTS

Transgene expression of β-galactosidase

Expression of the reporter gene was consistently detected at the periventricular areas since 6 hours to 7 days after gene transfer (Fig. 1A). X-Gal staining was not



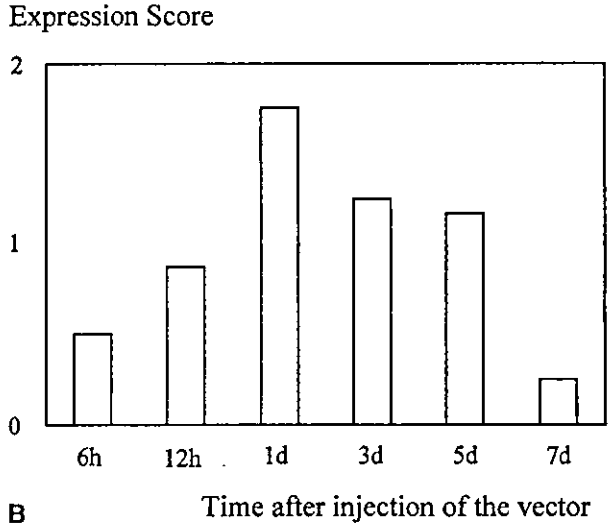


FIG. 1. Transgene expression in rat brains after gene transfer. (A) Coronal section of the brain 1 day after the ischemic insult. The transgene (β-galactosidase) was expressed at the lateral ventricles as a blue color with X-Gal staining. (B) Transgene expression was assessed at the ventricle 6 hours (n = 1), 12 hours (n = 2), 1 day (n = 3), 3 days (n = 2), 5 days (n = 3), and 7 days (n = 1) after injection of AdlacZ, and mean values are shown. The expression was observed at the ependyma as early as 6 hours after gene transfer of β-galactosidase, and peaked at day 1 followed by gradual decreases.

observed in the cortex, as reported previously (Kumai et al., 2003). The time course of semiquantitative analysis for transgene expression at the periventricular area is shown in Fig. 1B. Transgene expression was observed at the ependyma as early as 6 hours after gene transfer, and peaked at day 1 followed by gradual decreases.

Measurement of 7ND

Values of ELISA for 7ND in the CSF from the Ad7ND group are shown in Fig. 2. A marked amount of 7ND was detected 6 hours after gene transfer (8,010 ± 1,965 pg/mL). Amounts of dominant negative MCP-1 were significantly increased at day 1 and day 5 (43,600 ± 866 and 19,467 ± 5,105 pg/mL, respectively) as compared with those seen at 6 hours ($P < 0.01$). In the AdlacZ group and in normal rats, 7ND was undetectable in the CSF.

Physiologic variables

Physiologic variables before and after ischemia in AdlacZ and Ad7ND groups are shown in Table 1. There were no significant differences in physiologic variables before and after ischemia between the two groups. Blood flow to the cortex on the occlusion side began to decrease within 10 minutes after focal ischemia and lasted for more than 60 minutes. CBF reductions in AdlacZ and Ad7ND group at 60 minutes were −62% ± 17% and −69% ± 10%, respectively. Changes in CBF were not significantly different between the two groups (Fig. 3).

Infarct volume and leukocyte infiltration

Infarct volume in the AdlacZ and Ad7ND groups is shown in Fig. 4. Infarct volume in the Ad7ND group (75 ± 13 mm^3) was significantly smaller (by 28%) than that observed in the AdlacZ group (104 ± 22 mm^3, $P < 0.05$). Numbers of total and mononuclear leukocytes in the vessels at the infarct area in the Ad7ND group were 48.3 ± 32.9/cm^2 and 35.3 ± 27.7/cm^2, and were significantly smaller than those measured in the AdlacZ group (143.8 ± 72.1/cm^2 and 89.6 ± 40.9/cm^2, respectively; $P < 0.05$) (Fig. 5). Numbers of polynuclear leukocytes in the vessels at the infarct area tended to be lower in the Ad7ND group (13.0 ± 10.1/cm^2) than in the AdlacZ group (54.2 ± 13 /cm^2, $P = 0.07$).

TABLE 1. *Physiologic variables*

	AdlacZ (n = 5)	Ad7ND (n = 5)
At rest		
Body weight (g)	368 ± 22	366 ± 38
Head temperature (°C)	35.8 ± 0.2	35.8 ± 0.1
Rectal temperature (°C)	36.9 ± 0.1	37.1 ± 0.1
MABP (mm Hg)	181 ± 21	178 ± 13
Ht (%)	44.8 ± 0.8	44.0 ± 0.7
pH	7.42 ± 0.03	7.41 ± 0.04
$Paco_2$ (mm Hg)	37.2 ± 2.3	37.9 ± 3.7
Pao_2 (mm Hg)	131 ± 15	133 ± 11
BS (mg/dL)	139 ± 4	143 ± 13
One hour after dMCAO		
Head temperature (°C)	35.9 ± 0.2	35.9 ± 0.3
Rectal temperature (°C)	36.9 ± 0.2	37.1 ± 0.2
MABP (mm Hg)	203 ± 41	192 ± 19
Ht (%)	44.4 ± 0.5	43.6 ± 1.1
pH	7.40 ± 0.19	7.41 ± 0.02
$Paco_2$ (mm Hg)	36.0 ± 2.8	36.4 ± 3.2
Pao_2 (mm Hg)	133 ± 9	123 ± 8
BS (mg/dL)	122 ± 7	123 ± 10

Values are mean ± SD.
Ht, hematocrit; BS, blood sugar.

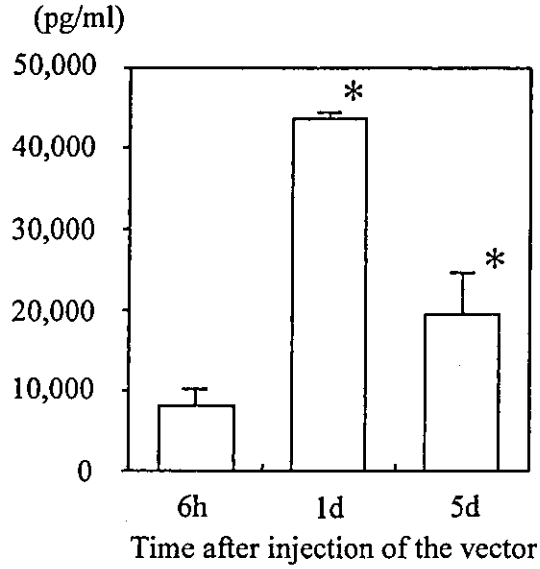


FIG. 2. Amount of dominant negative MCP-1 (7ND) in the CSF after gene transfer. A marked amount of 7ND was detected 6 hours after gene transfer. The level of 7ND at days 1 and 5 was significantly increased as compared with that at 6 hours ($P < 0.01$). Values are mean ± SD. *$P < 0.01$ versus 6 hours.

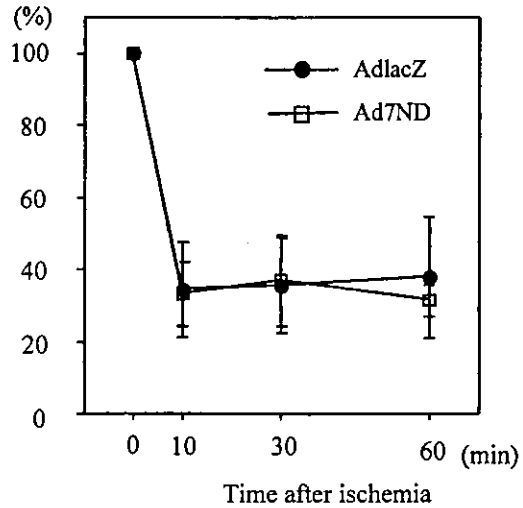


FIG. 3. Changes in cerebral blood flow at parietal cortices during distal middle cerebral artery occlusion. Blood flow to the cortex on the occlusion side began to decrease within 10 minutes after focal ischemia and lasted for more than 60 minutes. Changes in CBF were not significantly different between the two groups. Values are mean ± SD.

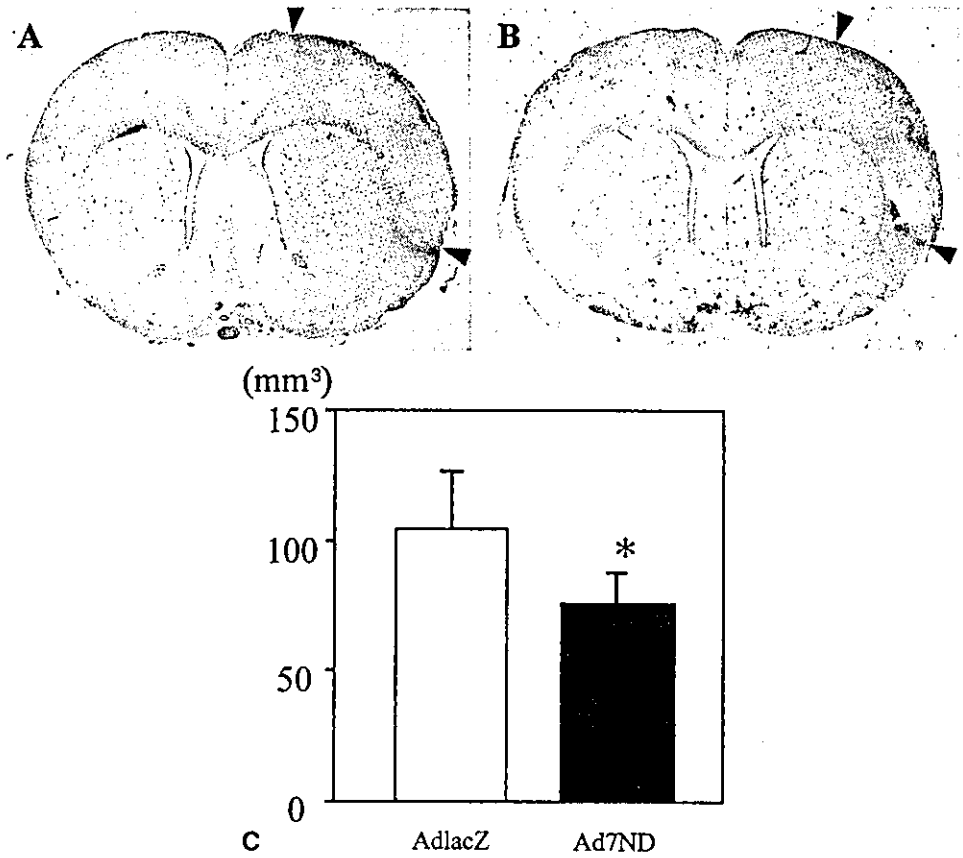


FIG. 4. Brain infarction after gene transfer. Hematoxylin-eosin staining of rat brains 5 days after brain ischemia and gene transfer (**A, B**). The infarct area (between the two arrowheads) in the AdlacZ group (**A**) was larger than that of the Ad7ND group (**B**). Infarct volume in the Ad7ND group (75 ± 13 mm^3) was significantly smaller than that observed in the AdlacZ group (104 ± 22 mm^3, $P < 0.05$, **C**). Values are mean ± SD. *$P < 0.05$ versus AdlacZ.

Immunohistochemistry of macrophage

ED1-positive cells were predominantly located in the border of the ischemic area (Figs. 6A and 6B, brown). There were fewer ED1-positive cells in the infarct area of the Ad7ND group (475.2 ± 125.5/mm^2) than in the AdlacZ group (671.8 ± 125.5 /mm^2, $P < 0.05$) (Fig. 6E).

DISCUSSION

In this study, gene transfer with adenoviral vectors into the lateral ventricle provided marked expression and release of transgene products in the CSF as early as 6 hours after gene transfer. Gene transfer of dominant negative MCP-1 reduced infarct volumes even when vectors were delivered after induction of focal brain ischemia, and the reduction of infarct size was associated with attenuations of both macrophage/monocyte and leukocyte infiltrations. Therefore, we clearly demonstrated the protective effect of anti–MCP-1 gene therapy against focal brain ischemia.

MCP-1 is involved in several inflammatory diseases, including rheumatoid arthritis (Koch et al., 1992), nephritis (Panzer and Stahl, 1999), infections (Dawson et al., 2000; Sato et al., 1999), and atherosclerosis (Egashira, 2003; Yla-Herttuala et al., 1991). These data indicate that MCP-1 is one of the major proinflammatory cytokines. In the central nervous system, MCP-1 was detected in the serum and CSF of patients with multiple sclerosis and the ischemic stroke (Franciotta et al., 2001; Losy and Zaremba, 2001). Previous studies have reported that MCP-1 deficiency in genetically altered mice and the nonpeptide C-C chemokine receptor antagonist TAK-779 reduced infarct volume and macrophage accumulations in the stroke model (Hughes et al., 2001; Takami et al., 2002), and that anti–MCP-1-neutralizing antibody attenuated *N*-methyl-D-aspartate–induced brain injury in the striatum and hippocampus (Galasso et al., 2000). These lines of evidence suggest that inhibition of MCP-1 may be neuroprotective in the setting of brain ischemia.

Recently, anti–MCP-1 gene transfer with dominant negative gene has shown protective effects in several experimental models, including rat vascular injury induced by chronic blockade of nitric oxide synthases (Egashira et al., 2000), atherosclerosis in apolipoprotein E–knockout mice (Ni et al., 2001), restenosis after coro-

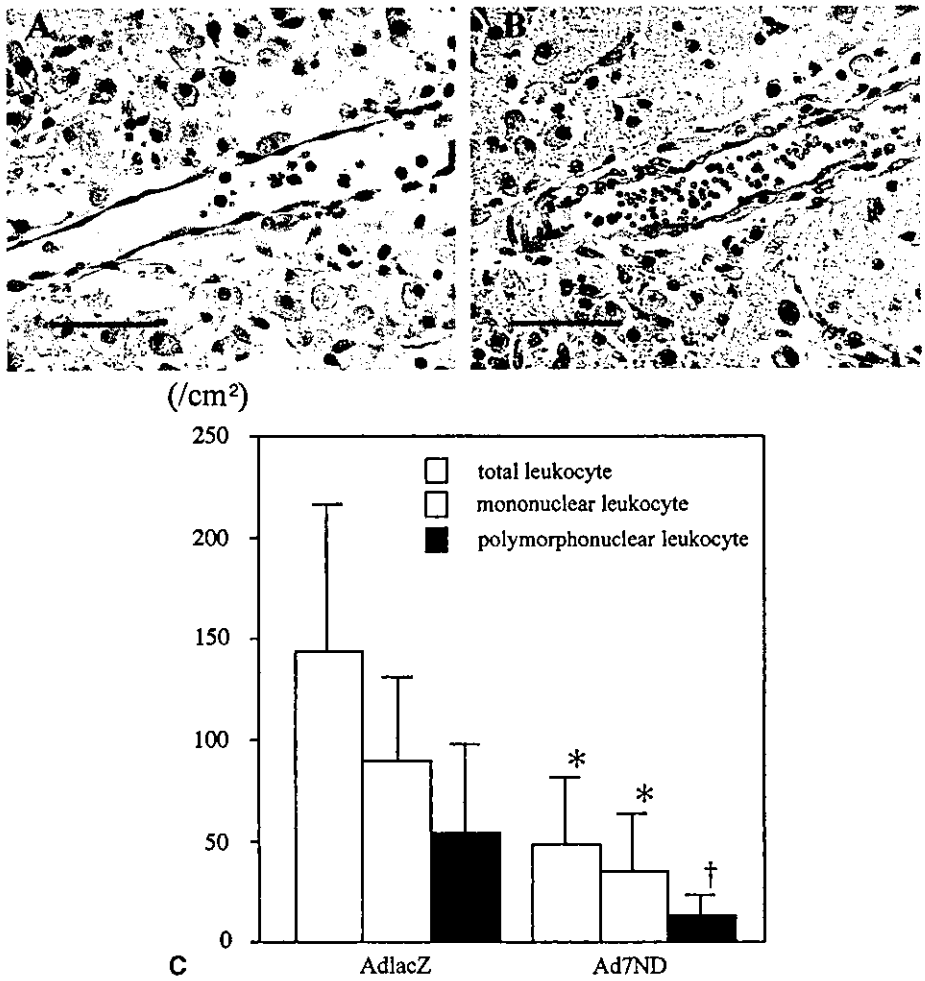


FIG.5. Leukocyte infiltration at the ischemic area after gene transfer. Hematoxylin-eosin staining of rat brains 5 days after brain ischemia and gene transfer (**A, B**; scale bar = 50 μm). Leukocyte counts in the vessels at the ischemic area were higher in the AdlacZ group (**A**) than in the Ad7ND group (**B**). Numbers of total and mononuclear leukocytes were significantly lower in the Ad7ND group than in the AdlacZ group (**C**). Numbers of polynuclear leukocytes tended to be lower in the Ad7ND group than in the AdlacZ group (†$P = 0.07$). Values are mean ± SD. *$P < 0.05$ versus AdlacZ.

nary intervention in rats and monkeys (Usui et al., 2002), pulmonary hypertension in rats (Ikeda et al., 2002), renal ischemia reperfusion injury in mice (Furuichi et al., 2003), and myocardial infarction in mice (Hayashidani et al., 2003). Our study clearly shows a protective effect of dominant negative MCP-1 gene transfer in the focal ischemia model. Thus, anti–MCP-1 gene transfer may be useful in the treatment of acute brain ischemia.

One of the major mechanisms of neuroprotection by anti–MCP-1 gene therapy is the inhibition of monocyte/macrophage infiltrations. MCP-1 is involved in monocytic recruitment in several inflammatory setting *in vivo,* including the brain (Bell et al., 1996; Lu et al., 1998). Forty-eight hours after focal brain ischemia, transgenic mice overexpressing MCP-1 had more monocyte/macrophage infiltrations than control mice (Chen et al., 2003). We showed that 7ND gene transfer significantly attenuated monocyte/macrophage activity 5 days after stroke, which was associated with smaller infarcts as compared with the control group. Therefore, the beneficial effect appeared to be attributable to inhibition of monocyte/macrophage recruitment and activation.

We showed that anti–MCP-1 gene transfer significantly attenuated total and mononuclear leukocyte counts and tended to attenuate polynuclear leukocyte counts in the vessels at the infarct area. In a previous study, MCP-1–transgenic mice were reported to have a trend toward an increase in neutrophils in a distinct area surrounding intraparenchymal blood cells in the ischemic brain (Chen et al., 2003). Furthermore, anti–MCP-1-neutralizing antibody prevented neutrophil influx in newborn hyperoxia-exposed rats, and had a trend toward a reduction of cytokine-induced neutrophil chemoattractant (Vozzelli et al., 2003). Thus, the decrease in leukocyte infiltrations may also have contributed to neuroprotection in our study.

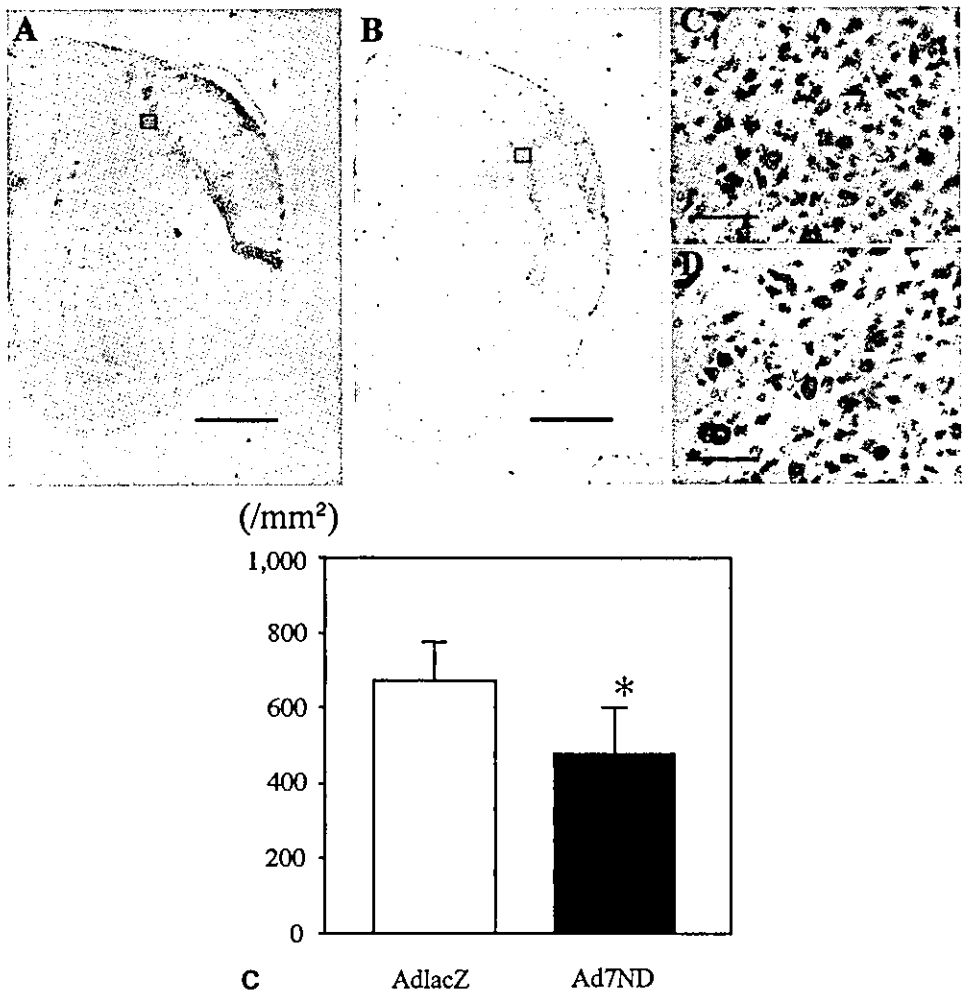


FIG. 6. Macrophage infiltration 5 days after brain ischemia and gene transfer. Sections were immunochemically stained with antimacrophage Ab, ED1 (**A–D**). Microscopic views of the brain in the AdlacZ group (**A**) or Ad7ND group (**B**) are shown in low-power fields (scale bar = 2 mm). The squares located in the border of ischemia are high-power-field views (**C, D**; scale bar = 50 μm). Macrophage counts at the border of ischemia were lower in the Ad7ND group (**D**) than in the AdlacZ group (**C**). Numbers of macrophages at the ischemic area were significantly lower in the Ad7ND group than in the AdlacZ group (**E**). Values are mean ± SD. $^*P < 0.05$ versus AdlacZ.

Proinflammatory cytokines, such as interleukin 1β (IL-1β) and tumor necrosis factor-α (TNF-α), are induced in response to brain ischemia (Arvin et al., 1996, Rothwell and Luheshi, 2000). Several studies have reported that injection of IL-1β in brain ischemia exacerbates brain damage (Loddick and Rothwell, 1996; Yamasaki et al., 1995). In contrast, IL-1β blockade and IL-1 deficiency cause a significant reduction of ischemic damage (Boutin et al., 2001; Yamasaki et al., 1995). TNF-α is another important mediator after brain ischemia, and may act as a pleiotropic peptide to elicit the production of IL-1β and adhesion molecules in brain ischemia (Ellison et al., 1999). Adhesion molecules are induced by IL-1β and TNF-α (Hess et al., 1994) and play a pivotal role in leukocyte infiltrations (Danton and Dietrich, 2003). IL-1β was induced by MCP-1 in human monocytes (Jiang et al., 1992), and the induction after focal brain ischemia was significantly reduced in MCP-1–knockout mice (Hughes et al., 2001). 7ND gene transfer significantly reduced several cytokines, including IL-1β, TNF-α, interleukin 6, transforming growth factor β, and MCP-1 in aorta (Inoue et al., 2002, Ni et al., 2004) and TNF-α, transforming growth factor β, and MCP-1 in ischemic heart from 1 to 7 days after myocardial infarction (Hayashidani et al., 2003). Therefore, the anti–MCP-1 approach appears to suppress the induction of proinflammatory cytokines and thereby inhibit the upregulation of adhesion molecules and inflammatory cell infiltrations, which would result in attenuation of tissue injury by brain ischemia.

In this study, 7ND gene transfer into cerebral ventricle reduced the infarct volume and provided marked expression of transgene in the CSF as early as 6 hours after gene transfer. Administration of the adenovirus into the lateral ventricle has produced extensive expression in the ependymal cells (Bajocchi et al., 1993; Ooboshi et al.,

1995), and preinjection of adenovirus carrying 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. In other studies using 7ND gene transfer, intramuscular transfection of the 7ND resulted in marked secretions of 7ND protein into the circulating blood, which bound to the MCP-1 receptor on monocytes or target cells and, thus, achieved an effective blockade of MCP-1 activity in remote organs (Egashira, 2003). Although 7ND gene transfer to skeletal muscle has obstacles of the blood–brain barrier, the delivery method may be applicable to ischemic stroke where the barrier is disturbed.

In our study, gene transfer initiated 90 minutes after stroke reduced the volume of brain infarction. Although several studies reported protective effects of postischemic gene therapy (Hayashi et al., 2001; Hoehn et al., 2001; Lawrence et al., 1997; Shimazaki et al., 2000; Zhang et al., 2002), those studies were examined with the transient brain ischemia model, and the report that achieved reductions of brain infarct volume in the permanent ischemia model was limited. The therapeutic time window of postischemic gene transfer lasted up to 90 minutes in our study, but it did not by 150 minutes after ischemia in another study (Zhang et al., 2002). Therefore, it is necessary to examine a longer therapeutic time window in our model. Combination of gene therapy and protein may increase the therapeutic time window, because neurotrophic peptide may have a neuroprotective effect up to 150 minutes after ischemia (Zhang et al., 2001).

The focal brain ischemia produced by photochemical occlusion of the distal MCA of SHR provides small variations in infarct volume without extensive surgery (Yao et al., 2003). However, one of drawbacks in our model is difficulty in the assessment of neurologic function, because this model shows small neurologic deficit due to the relatively confined infarction to the cortex. Although the pathologic features of our model are similar to those of other models (Yao et al., 1996) and suitable for examination of cytoprotective drugs (Cai et al., 1998), further examination, including the use of primate models, is inevitable before clinical trials can be started.

In conclusion, postischemic gene transfer of dominant negative MCP-1 protects against focal brain ischemia, which is related to the reduction of both macrophage/monocyte and leukocyte infiltrations. Anti–MCP-1 gene therapy may be a promising approach for the treatment of brain ischemia.

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□ CASE REPORT □

Recurrent Small-Artery Disease in Hyperhomocysteinemia: Widowers' Stroke Syndrome?

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Abstract

Hyperhomocysteinemia is thought to cause ischemic strokes. We report two middle-aged widowers with frequent recurrences of small-artery strokes, two capsular infarcts and a thalamic hemorrhage in one patient, and two thalamic and pontine infarcts in the other. Blood tests following the final stroke showed hyperhomocysteinemia and methylenetetrahydrofolate reductase C677T gene mutation, with low concentration of vitamin B6. Multivitamin supplementation normalized plasma homocysteine levels in both patients. Hyperhomocysteinemia is treatable; therefore, serum homocysteine should be measured as a potential risk factor for stroke recurrence in relatively young patients with recurrent small-artery infarctions or hemorrhage, especially those with insufficient lifestyle factors.
(Internal Medicine 43: 869–872, 2004)

Key words: homocysteine, methylenetetrahydrofolate reductase, lacunar infarction, cerebral hemorrhage, stroke prevention

Introduction

According to recent meta-analyses, elevation of plasma homocysteine (Hcy) level is associated with an increased risk of ischemic stroke (1, 2). Among stroke subtypes, hyperhomocysteinemia (hyper-Hcy) predisposes to large-artery atherosclerosis including carotid stenosis (3–5). Patients with small-artery infarction are also reported to have higher serum Hcy levels than control patients (4–6). Although hyper-Hcy does not seem to associate with embolic stroke (4, 5), it conveys an independent risk for left atrial thrombus formation in stroke patients with nonvalvular atrial fibrillation (7). The relationship between hyper-Hcy and hemorrhagic stroke has not been clarified. Boysen et al (8) recently demonstrated that elevated serum Hcy is an independent risk factor for recurrent ischemic strokes based on a prospective study of 1,039 patients.

Methylenetetrahydrofolate reductase (MTHFR) serves as an enzyme for conversion of dietary folate to 5-methyltetrahydrofolate and a methyl donor requires the remethylation of Hcy to methionine *in vivo* (1). Although the MTHFR C677T gene mutation, a common polymorphism in this gene, increases plasma Hcy levels (9), the mutation has not been reported as a consistent risk factor for stroke (1, 10). MTHFR TT genotype seems to be an independent risk factor for silent brain infarction and white matter lesions in the general Japanese population (11).

We report here two middle-aged men with hyper-Hcy and MTHFR TT genotype who had repeated lacunar infarctions and ganglionic hemorrhage under good management of other risk factors except for hyper-Hcy. Multivitamin supplementation was successful for management of hyper-Hcy.

For editorial comment, see p 769.

Case Reports

Patient 1

A 53-year-old normotensive clerk developed strokes 3 times during 5 months, and was admitted to our hospital for the third stroke. He had a 30-year history of smoking before quitting after the first stroke. After the death of his wife 4 years previously, he lived alone and often dined on box lunches and noodles. Right hemiparesis was the symptom of the former two ischemic strokes, which occurred at a one-month interval. Left corona radiata and left basal ganglia

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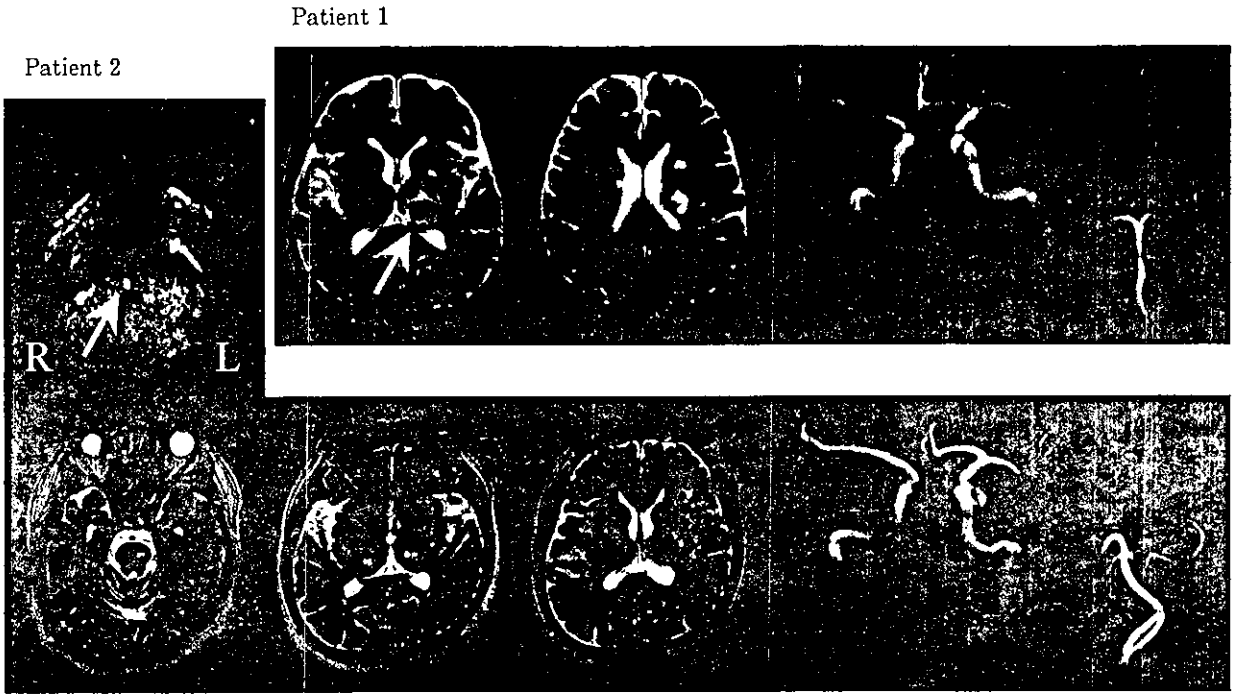

Figure 1. Cranial MRI and MRA on admission following the final stroke. T2-weighted image reveals a left thalamic hematoma in Patient 1 (arrow). Diffusion-weighted image reveals a fresh infarct in the right tegmentum of the pons in Patient 2 (arrow).

were the culprit lesions. Oral administration of aspirin (81 mg/day) was started after the first stroke, and ticlopidine (200 mg/day) was added after the second. One evening 4 months after the second stroke, he suddenly noticed dysesthesia in his right hand, and the symptom spread into the right side of his body within an hour. When he visited our hospital the next morning, blood pressure was 140/82 mmHg, and pulse rate was regular at 60/min. He had a moderate paresis of the right limbs with accelerated deep tendon reflexes. A new neurological sign was the decreased perception of touch, pain and temperature on the right side of the face and right limbs.

Cranial MRI revealed a fresh hematoma in the left thalamus and small old infarcts in the left basal ganglia and corona radiata (Fig. 1). Cranial MRA did not show stenosis of arteries. On blood testing, parameters for common diseases including lipids and hemoglobin A1c were normal except for increased level of Hcy (22.5 μmol/*l*). Among serum vitamins, B12 (320 ng/*l*) and folate (3.3 μg/*l*) were within normal levels, and B6 was slightly decreased (5.9 μg/*l*). Polymerase chain reaction DNA amplification was performed using whole blood lymphocytes, and MTHFR TT genotype was documented. Cardiac investigations and hemostatic tests did not show any evidence for embolic stroke. Ambulatory blood pressure monitoring showed normotension without morning surge.

Oral supplementation of vitamin B6 (30 mg/day) did not change the plasma level of Hcy (Fig. 2). Addition of vitamin B12 (1.5 mg/day) and folate (15 mg/day) brought the Hcy level down to normal. There have been no recurrent strokes for a period of more than a year.

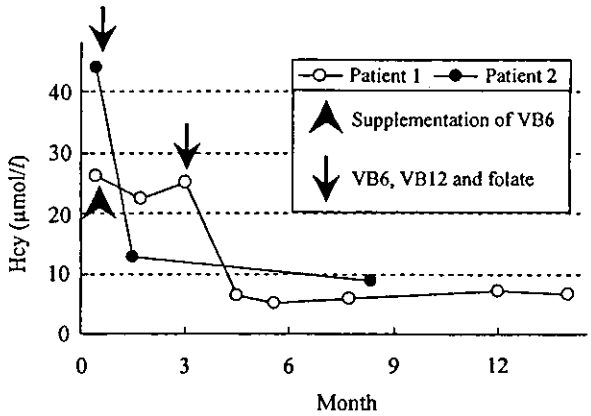

Figure 2. Changes in plasma homocysteine level following vitamin supplementation.

Patient 2

A 59-year-old widower, who lived alone, with infrequent

intake of vegetables, daily alcohol consumption of 500 ml of spirits, and a previous history of smoking, felt a tingle in his left arm when he awoke one morning, and visited our hospital. He had repeated pure sensory strokes due to the left thalamic infarction at 6 years and 2 years before; in addition to silent infarcts in the left pons and right thalamus indicated 7 years before. Oral administration of ticlopidine (200 mg/day) was continued after the first stroke. Although he was hypertensive in the previous medical examination, he kept normotensive without use of antihypertensives after the first stroke. On admission, his blood pressure was 150/80 mmHg, and pulse rate a regular 80/min. He was neurologically intact except for dysesthesia in the left arm and chronic hypesthesia in the right limbs. Cranial MRI delineated a fresh infarct in the right tegmentum of the pons and small old infarcts in the left pons, both thalami, and both basal ganglia (Fig. 1). Large arteries were normal on cranial MRA. On blood test, Hcy level was high (44.1 μmol/*l*), concentrations of vitamin B6 (2.1 μg/*l*) and folate (2.3 μg/*l*) were low, and vitamin B12 was within the normal level (330 ng/*l*). Parameters for common diseases including lipids and hemoglobin A1c were normal. Genetic examination revealed MTHFR TT genotype. Cardiac investigations and hemostatic tests were intact. Ambulatory blood pressure monitoring showed normotension without morning surge.

Oral supplementation of vitamin B6 (30 mg/day), vitamin B12 (1.5 mg/day), and folate (15 mg/day) brought the Hcy level down to normal (Fig. 2). He has been free from stroke for a period of more than a year.

Discussion

The main aspects of this report are that middle-aged men with uncontrolled hyper-Hcy and MTHFR TT genotype had repeated small-artery infarctions and hemorrhage at short intervals, and further that multivitamin supplementation of B6, B12, and folate returned the relatively high plasma Hcy levels to normal.

Although plasma Hcy levels were not measured before the final stroke, the levels might have been high before the first stroke because of the existence of MTHFR gene mutation. A deficiency of the dietary vitamins required for metabolism of Hcy in vivo might have resulted from the insufficiencies of their dietary habits, which are quite common for middle-aged Japanese men living without wives. Population-based studies reported that plasma Hcy levels were positively related with male gender and alcohol consumption (12, 13). Previous smoking habit and hypertension were other possible risk factors for the strokes. However, because these two factors were well controlled after the first strokes, hyper-Hcy seemed to be an essential risk factor for stroke recurrence in the present patients. Hcy levels went far beyond the reported mean values for 1,487 stroke patients (13.51 μmol/*l* versus 11.07 μmol/*l* for nonstroke subjects) in a meta-analysis (1).

Among the three small-population studies regarding the positive association of hyper-Hcy with lacunar infarction (4–6), two indicated a weak association (4, 6), while the other indicated that the association was a little stronger than that with large-artery atherosclerosis (5). In addition, hyper-Hcy has been suggested to associate with subcortical vascular encephalopathy (14, 15). Although the pathology of deep perforating arteries and subcortical microvessels may differ, these findings suggest that hyper-Hcy contributes to small artery diseases in a fashion different from atherosclerosis, possibly including the formation of microatheroma. A positive relationship between hyper-Hcy and hemorrhagic stroke has been reported in children and infants (16, 17), but not in an adult population. Because ganglionic hemorrhage usually results from the same vascular pathology with lacunar infarction, there might also be a positive association between hyper-Hcy and ganglionic hemorrhage. For Patient 1, intensive antiplatelet therapy might have been a trigger for hemorrhagic stroke. Thus, measurement of plasma Hcy level, and if needed its normalization, appears to be necessary for cryptogenic lacunar infarction before antithrombotic medication.

According to the meta-analysis (2), lowering the Hcy level by 3 μmol/*l* from current levels would reduce the risk of stroke by about 24% (15 to 33%). From the therapeutic results of Patient 1, a single vitamin supplementation is not sufficient, and multivitamin supplementation of B6, B12, and folate seems to be necessary regardless of baseline serum levels. Two large clinical trials on the prevention of recurrent stroke, Vitamin Intervention for Stroke Prevention (VISP) (18) and Vitamins to Prevent Stroke (VITATOPS) (19), are underway.

In conclusion, hyper-Hcy may be a potential risk factor for recurrent small-artery infarctions and hemorrhage. Serum Hcy should be measured for relatively young patients with recurrent small-artery strokes, especially those with insufficiencies of lifestyle factors; thus the chance of management for this treatable risk factor will not be lost.

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Brain infarction associated with antiphospholipid antibody syndrome caused by paradoxical embolism through patent foramen ovale

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Sirs: Antiphospholipid antibody syndrome (APS) is well known as a disorder that likely causes various thrombotic events, including brain infarction [2, 6]. However, it is often difficult to identify the precise mechanism of the brain infarction associated with APS in each case, because of its complexity of pathophysiology, which may be derived from the heterogeneity of antiphospholipid antibodies, including lupus anticoagulants, anti-cardiolipin antibodies, and anti-β_2-glycoprotein I (β_2-GPI) antibodies [2, 4, 8]. The heterogeneity may produce dysfunction of several factors, namely vascular endothelial cells [1], platelets [2, 10], and anticoagulant proteins, such as protein S and protein C [2, 9]. Brain infarction can be classified into three different subtypes according to its mechanism: atherothrombotic, cardioembolic, and lacunar infarction. Endothelial injury of cerebral arteries by antiphospholipid antibodies probably leads to *in situ* atherothrombosis and that of the cardiac valves causes cardiogenic embolism by production of nonbacterial vegetation on the valves [5]. Hypercoagulability caused by dysfunction of protein S or protein C may induce the formation of thrombus in the left atrium especially when there is mitral valve stenosis or atrial fibrillation, thereby causing cardiogenic embolism. Thus, APS can theoretically cause all subtypes [14]. Several reports have suggested that cardiogenic embolism is frequently involved in brain infarction with APS [3, 14]. We present a case that developed a brain infarct caused by paradoxical embolism through a patent foramen ovale (PFO) during the course of APS.

A 42-year-old woman was admitted to our hospital with complaints of pain of bilateral lower extremities and a sudden-onset left-sided homonymous hemianopsia. She had been diagnosed as primary APS by recurrent miscarriages and the persistent presence of lupus anticoagulant and anti-β_2-GPI antibody at age 35 years. However, she had received no medication including anticoagulation until the admission. On the admission, blood pressure was 142/92 mmHg and pulse was 72 per min and regular. Her legs were bilaterally swollen with dilatation of superficial veins. Neurologically, a left-sided homonymous hemianopsia was present. Coagulation studies showed that thrombin-antithrombin III complex (6.9 ng/ml) and D-dimer (20 ng/ml) were elevated. Magnetic resonance venography of the lower extremities showed that the bilateral femoral and left saphenous veins were occluded (Fig. 1A). Intracranial magnetic resonance imaging showed a brain infarct in the right occipital lobe (Fig. 1B). Magnetic resonance angiography of the intracranial arteries and Doppler ultrasound sonography of carotid and vertebral arteries showed no stenotic lesion. Transesophageal echocardiogram demonstrated the existence of PFO, without atrial septal

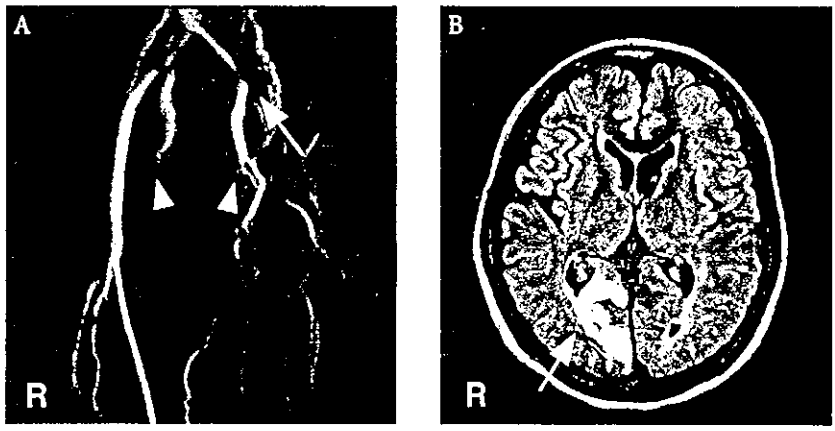

Fig. 1 Magnetic resonance imaging. **(A)** Magnetic resonance venography of lower extremities (ECG-gated two-dimensional time-of-flight) showed that bilateral femoral (arrowhead) and the left saphenous (arrow) veins were occluded with dilatation of collateral veins. **(B)** Intracranial fluid attenuated inversion recovery imaging showed a high intensity area in the right occipital lobe (arrow)

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aneurysm. Microbubbles through the interatrial right-to-left shunt were induced and detected by the Valsalva-maneuver (Fig. 2). The degree of shunting was small according to existing criteria [11, 13]. All these findings were consistent with the criteria of paradoxical embolism [12]. An anticoagulation therapy with the low molecular-weight heparin was performed. Both the deep vein thrombosis (DVT) and the left-sided homonymous hemianopsia gradually improved. A strict anticoagulation therapy with warfarin was continued after discharge.

We have concluded that a brain infarct in the present case was caused by paradoxical embolism through the PFO. There is an emerging literature regarding stroke and thrombophilia via paradoxical embolism [7, 11, 13, 15]. The present case is also consistent with the fact that cardioembolic infarction, including paradoxical embolism, often occurs in the posterior cerebral artery territory [13]. DVT is the most common manifestation among various thromboses in APS patients [5, 6, 14]. Since it is reported that about 20% of normal control subjects show some degree of the interatrial shunting [7, 15], it is possible that paradoxical embolism occurs in about 20% of APS patients. As far as we are aware, however, there have been no reports that emphasize the importance of paradoxical embolism as a cause of brain infarction with APS. We consider that a significant portion of APS patients that are diagnosed as cardiogenic brain infarction may include paradoxical embolism because of the close link between APS and DVT [5, 6, 14].

In conclusion, we describe a case of the brain infarction associated with APS where paradoxical embolism may have played an important role. We consider that paradoxical embolism is one important mechanism in brain infarction associated with APS, and the presence of DVT and PFO should therefore be checked.

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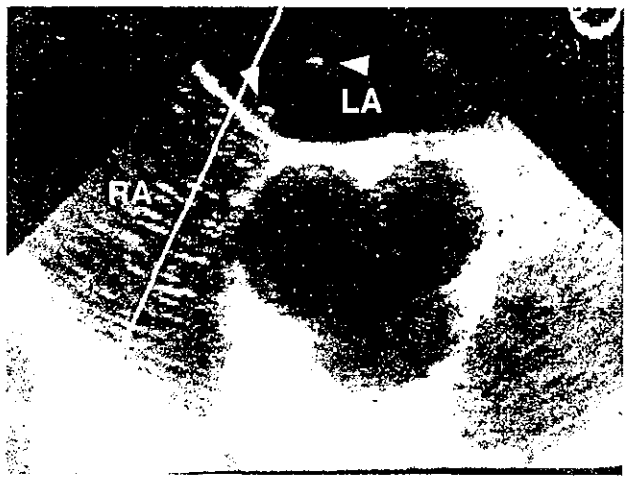

Fig. 2 Echocardiography. Transesophageal echocardiography detected microbubbles through the interatrial right-to-left shunt induced by the Valsalva-maneuver (arrowhead), indicating the presence of PFO

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Short communication

Renal cholesterol embolism in patients with carotid stenosis: a severe and underdiagnosed complication following cerebrovascular procedures

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Abstract

Here, we report two cases with rapidly progressive renal failure, caused by cholesterol crystal embolism (CCE), after an angiography for carotid artery stenosis. The diagnosis was determined by histological examination and from clinical symptoms, including livedo reticularis and eosinophilia. Neurologists and neuroradiologists tend to underdiagnose CCE, which results from the same atherosclerotic risk factors as cerebrovascular disease. We need to understand more about CCE and identify its unique clinical symptoms to enable an early diagnosis and treatment.

Keywords: Cholesterol crystal embolism; Carotid artery stenosis; Angiography; Carotid endarterectomy; Antithrombotic therapy; Renal infarction

1. Introduction

Cholesterol crystal embolism (CCE), the embolization of cholesterol crystals from atherosclerotic plaques of the aorta or large feeder arteries, is a significant complication of vascular procedures including angiography [1]. It causes multiorgan dysfunction including renal impairment, and results in a high 1-year mortality rate ranging from 64% to 87% [1–3]. Although CCE appears to be more common than previously reported [4,5], it still tends to be underdiagnosed and premortal diagnosis is often difficult [3]. Because patients with cerebrovascular diseases often had ulcerated plaques in the aorta [6,7], vascular procedures for such patients may risk CCE. Here, we report two cases of progressive renal failure after cerebral angiography. In addition to renal biopsy, eosinophilia and cutaneous changes were useful for the diagnosis of CCE as a cause of renal failure.

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2. Case report

2.1. Case 1

A 73-year-old man with hypertension, hyperlipidemia, and smoking habituation had a history of two minor brain infarctions and took 200 mg of ticlopidine hydrochloride daily. He also suffered from arteriosclerosis obliterance with intermittent claudication and ischemic heart disease. Because occlusive carotid lesions were identified on cervical MRA, performed to determine the cause of the ringing in his left ear for a year, he underwent a cerebral angiography for further examination (on day 1). It revealed a total occlusion of the right extracranial internal carotid artery (ICA) (Fig. 1A) and severe stenosis (73%) by NASCET's method of the left extracranial ICA (Fig. 1B). He visited our Cerebrovascular Center 14 days after the angiography (day 15) to receive management of the carotid lesions. On admission, blood pressure was 152/80 mm Hg and pulse rate a regular 70/min. Bruit was heard at the left neck. Retinal arterioles showed sclerotic changes without occlu-

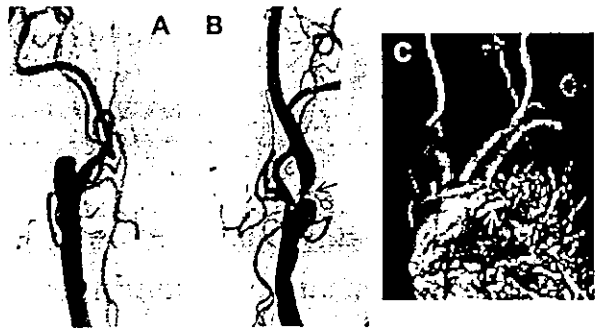

Fig. 1. Cerebrovascular images of case 1. (A) Right carotid arteriogram showed total occlusion of proximal ICA. (B) Left carotid arteriogram showed severe stenosis (73%) by NASCET's method of the proximal ICA. (C) 3D-MRA of the aortic arch showed ulcerative plaque (white arrow).

sion or intraarterial emboli. He did not have dermal disorders or neurological deficits. Brain MRI delineated multiple small infarcts in the bilateral basal ganglia.

Blood tests on day 16, revealed a total white blood cell count of 7100/μl (normal; ≦8000/μl) and eosinophil count of 277/μl (normal; ≦300/μl). Serum chemistry, immunology, and hemostatic examinations were normal including serum creatinine levels (1.2 mg/dl, normal; ≦1.2 mg/dl). Urinalysis revealed proteinuria at 3.5 g/day of protein excretion (normal; ≦120 mg/day). The eosinophil count and serum creatinine levels gradually increased without clinical symptoms or cutaneous changes, and on day 64 exceeded 600/μl and 3.0 mg/dl, respectively (Fig. 2A). He did not undergo catheter manipulations or have contrast agents during the period. Renal biopsy on day 67 detected cholesterol clefts in the lumen of the medium-sized artery (Fig. 3A), and his renal event was definitively diagnosed as CCE-induced ischemia. A potential source of the emboli is the aortic arch, and 3D-MRA showed irregularity of the aortic wall implicating the atheromatous plaque (Fig. 1C). Steroid treatment using 20 mg of oral prednisolone was started on day 77. On day 78, his serum creatinine level was 4.9 mg/dl, which then gradually returned to 3.4 mg/dl on day 87.

Although carotid endarterectomy (CEA) or endovascular surgery of his left ICA seemed to be the optimal strategy against the recurrence of a stroke, we decided against this because CCE might have recurred with this surgical procedure. We recommended him to continue use of oral ticlopidine.

2.2. Case 2

A 66-year-old man with hypertension, diabetes mellitus, smoking habituation, and a history of brain infarction

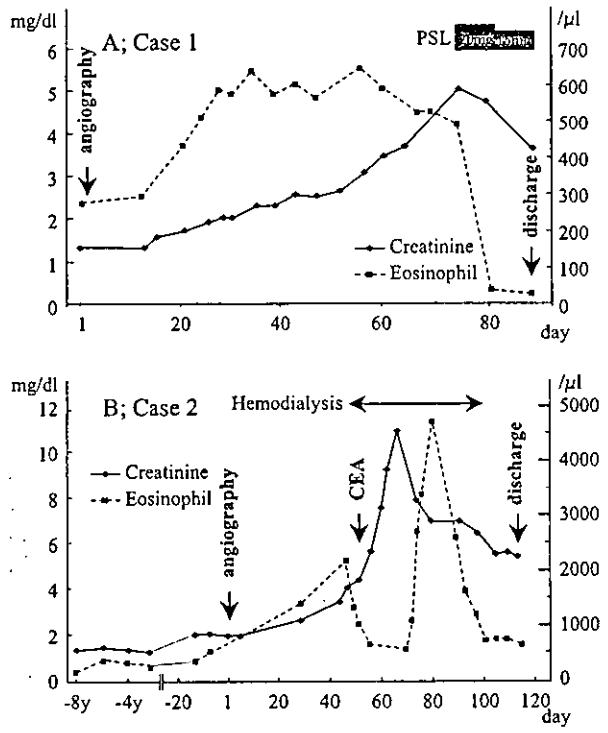

Fig. 2. Changes in serum creatinine level and eosinophil count.

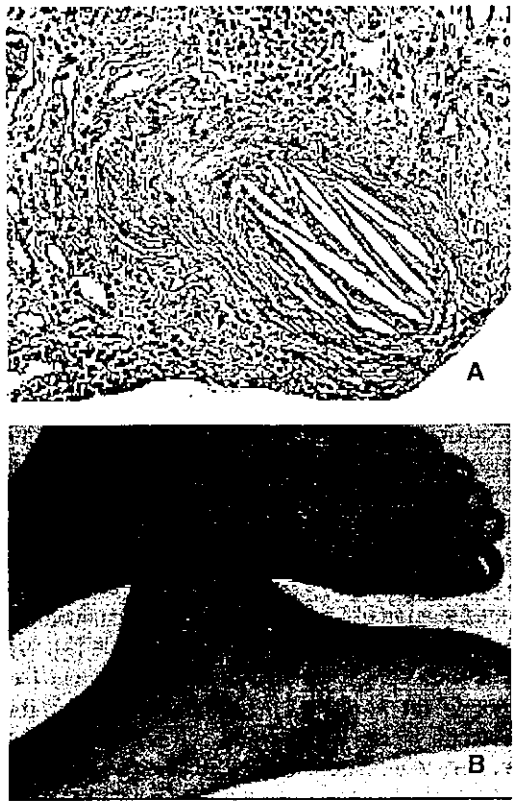

Fig. 3. Pathological findings indicative of cholesterol crystal embolism. (A) Right renal tissue specimen of patient 1 showed needle-shaped cholesterol clefts within the renal artery branch and localized neutrophilic infiltration. (B) Peripheral sites of the left foot of patient 2 showed cyanotic change and livedo reticularis (black arrows).

8 years earlier, entered our Cerebrovascular Center for management of progressive stenosis of the right ICA with ulcerative formation that had been determined by annual ultrasonography. His blood pressure was 138/86 mm Hg and pulse rate a regular 72/min. Physical and neurological examinations were normal except for bruit at the right neck. He had hypertensive nephrosclerosis with proteinuria and had elevated serum creatinine levels of 1.5 mg/dl 8 years earlier, which had risen to 2.1 mg/dl on admission (Fig. 2B). Blood urea nitrogen was 23 mg/dl (normal; ≦20 mg/dl) and creatinine clearance was 35.3 ml/min (normal; >90 ml/min). Serum chemistry was normal except for renal function, immunology, and hemostatic examinations.

Cerebral angiography showed ulcerative atheroma on the right ICA with a stenosis (67%) by NASCET's method as well as atherosclerotic changes in the multiple major arteries (Fig. 4A,B,C). Brain MRI revealed multiple small infarcts in the bilateral basal ganglia. One week after angiography, his creatinine level did not increase. When he returned to our center for surgery to the carotid lesion, 7 weeks after the angiography (on day 44), his blood pressure was elevated to 192/112 mm Hg and his feet showed livedo reticularis (Fig. 3B). Blood tests revealed serum creatinine was 3.4 mg/dl, urea nitrogen 37 mg/dl, and creatinine clearance 13.5 ml/min. His eosinophil count was increased to 2162/μl. Because CEA was reported to maintain renal function for patients with renal insufficiency [8], we performed CEA for his right ICA on day 54.

After CEA, serum creatinine levels were elevated to 7.5 mg/dl on day 58. He developed oliguria and respiratory failure due to pulmonary edema and pneumonia, and subsequently needed hemodialysis and mechanical ventilation. 3D-MRA of the aortic arch on day 8 showed irregularity of the wall suggesting atheromatous plaque (Fig. 4D). Although these clinical and laboratory findings strongly suggested renal impairment by CCE, we could not use steroid or immunosuppressive drugs because of severe pneumonia and a serum CRP of 27.33 mg/dl (normal; ≦0.30 mg/dl). He continued hemodialysis for 2 months, and his eosinophil count fluctuated during this period. After leaving hemodialysis on day 104, he did not suffer from any neurological deficits. He has continued to take oral ticlopidine to prevent the recurrence of a stroke.

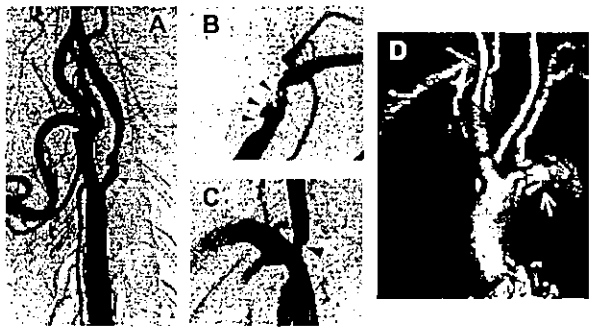

Fig. 4. Cerebrovascular images of case 2. (A) Right carotid arteriogram showed moderate stenosis (67%) by NASCET's method with ulcerative atheroma of the proximal ICA (black arrow heads). (B, C) Moderate stenosis of the left subclavian artery with ulceration (B) and right vertebral artery (C) were also detected by cerebral angiography (black arrow heads). (D) 3D-MRA of the aortic arch showed ulcerative plaque (white arrow).

3. Discussion

The main findings of this study are that cerebral angiography for patients with stenotic carotid lesion caused severe renal failure due to CCE and that it is critical to correctly diagnose this disease by observation of the specific symptoms, including eosinophilia, cutaneous manifestations, and acute hypertension.

Since Flory [9] first reported histopathology of CCE in 1945, it has been regarded as a unique systemic complication of atherosclerosis. Recently, invasive vascular procedures, such as angiography, cardiovascular surgery, and endovascular surgery, are known as precipitating factors [1–3]. Several interesting studies on CCE for the patients receiving cardiac catheterization have shown [5,10] that the frequency of CCE following left-heart catheterization was 1.4% in a prospective study with 1786 consecutive patients [5], and that visible atherothrombotic material was present in the backflow of cardiac catheters in 41 of 7621 patients (0.54%) [10]. Cerebral catheterization often needs a guiding catheter of smaller gauge than cardiac catheterization and is very unlikely to invade the ascending aorta, and accordingly not induce CCE as often as cardiac catheterization. In recent years, because patients with cerebrovascular disease have a greater chance to undergo endovascular surgery, the incidence of CCE has increased [1]. As neurologists and neuroradiologists, we are aware of the occurrence of ischemic stroke due to CCE [6,7,11], but often seem to overlook more common systemic events; i.e. renal failure and cutaneous lesion which occurred in 50% and 34% of the patients respectively, throughout the course of CCE [1–3].

Once it occurs, renal failure has much influence on the prognosis. Branches of the renal artery between 50 and 200 μm in diameter are frequently damaged and the size of both kidneys are reduced by ischemic infarction or patchy atrophy. Cutaneous lesions, such as cyanosis of the toes (blue-toe syndrome) or livedo reticularis of the lower limbs, usually occur before renal impairment, and reflect embolic disturbances of peripheral circulation [1]. In addition to these findings, nodules appear occasionally, as a result of the inflammatory reaction surrounding cholesterol crystals [1]. Thus, cutaneous manifestations seem to be important indicators of CCE. New-onset or accelerated hypertension is another frequent clinical observation, and may result from the release of excessive

renin from damaged kidneys [1]. Among these symptoms, eosinophilia is present in over 80% of patients with CCE [11]. It reflects an allergic reaction and generally lasts for only a few days. Fluctuation in our patient's eosinophil count over a period of weeks, might suggest recurrent shower embolism.

As therapeutic strategies for CCE, anticoagulant and thrombolytic therapies do not appear beneficial and may even cause CCE [1,2], even though we often perform these therapies for arteriosclerotic diseases including stroke and coronary arterial disease. Corticoteroid seems to be useful for reducing the inflammatory response, prevention of recurrent bouts of cholesterol embolism, and improvement of organ function [2,12]. However, a compromised state accompanied with CCE often prevents us from using a therapeutic dose of steroids. Plasma exchange has also been shown as an effective treatment to reduce blood viscosity by removing large-weighted molecules and improve blood flow [13]. Statin is another potential agent for the stabilization of cholesterol-rich plaques and preventing the recurrence of an embolism [2,14]. The effect of these medical treatments has not yet established, and requires further examination. Several studies have shown that renal dysfunction may not affect the perioperative complications in CEA. For example, Reil, et al. [8] proposed that CEA could be safely performed in patients even with chronic renal failure and serum creatinine levels in excess of 1.5 mg/dl. However in Case 2, renal failure developed rapidly following CEA, presumably partly because of perioperative hypotension and adverse effects to several drugs administered during the perioperative period. Thus, we need to carefully assess the surgical indication of CEA for patients with renal cholesterol embolism. Although severe renal damage occurred remotely after angiography in Case 2, we still think that angiography was a main etiological cause of his CCE, because CCE is mostly induced by invasive aortic procedures [1–3] and the course of renal dysfunction in CCE is usually subacute with a delay of weeks or months after the procedure [1].

In conclusion, we need to recognize CCE is an important complication of cerebrovascular interventions and be vigilant for the appearance of specific clinical symptoms of CCE after these procedures.

Acknowledgements

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