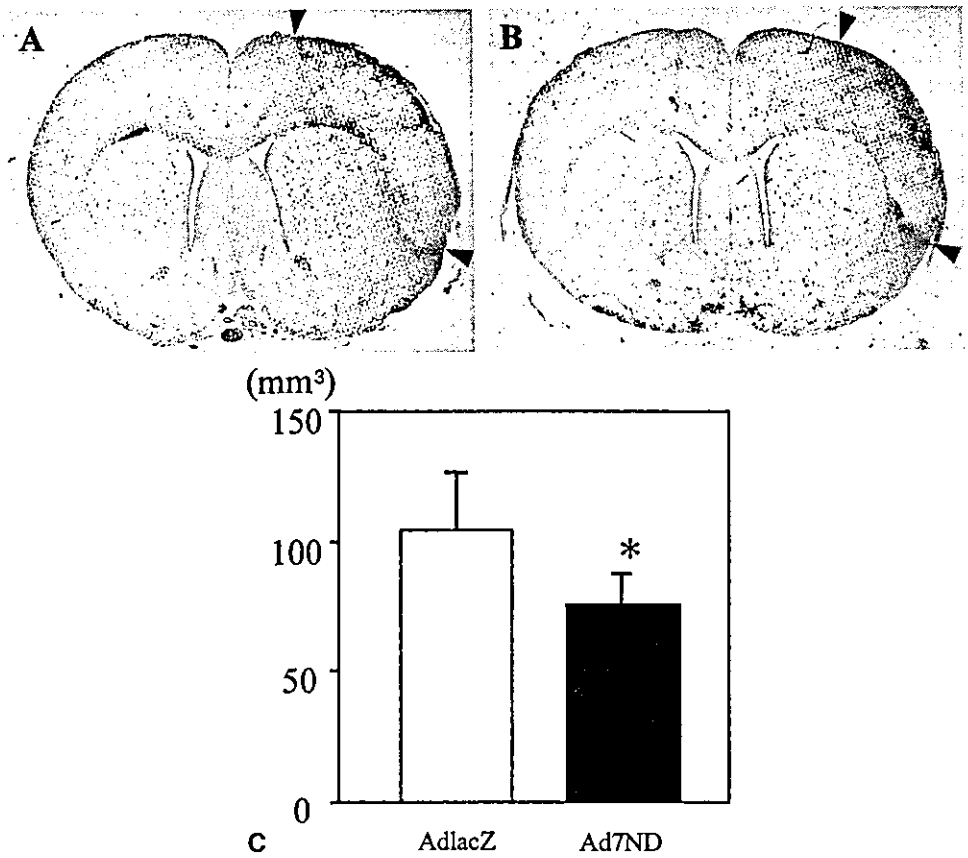

FIG. 4. Brain infarction after gene transfer. Hematoxylin-eosin staining of rat brains 5 days after brain ischemia and gene transfer (**A, B**). The infarct area (between the two arrowheads) in the AdlacZ group (**A**) was larger than that of the Ad7ND group (**B**). Infarct volume in the Ad7ND group (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-

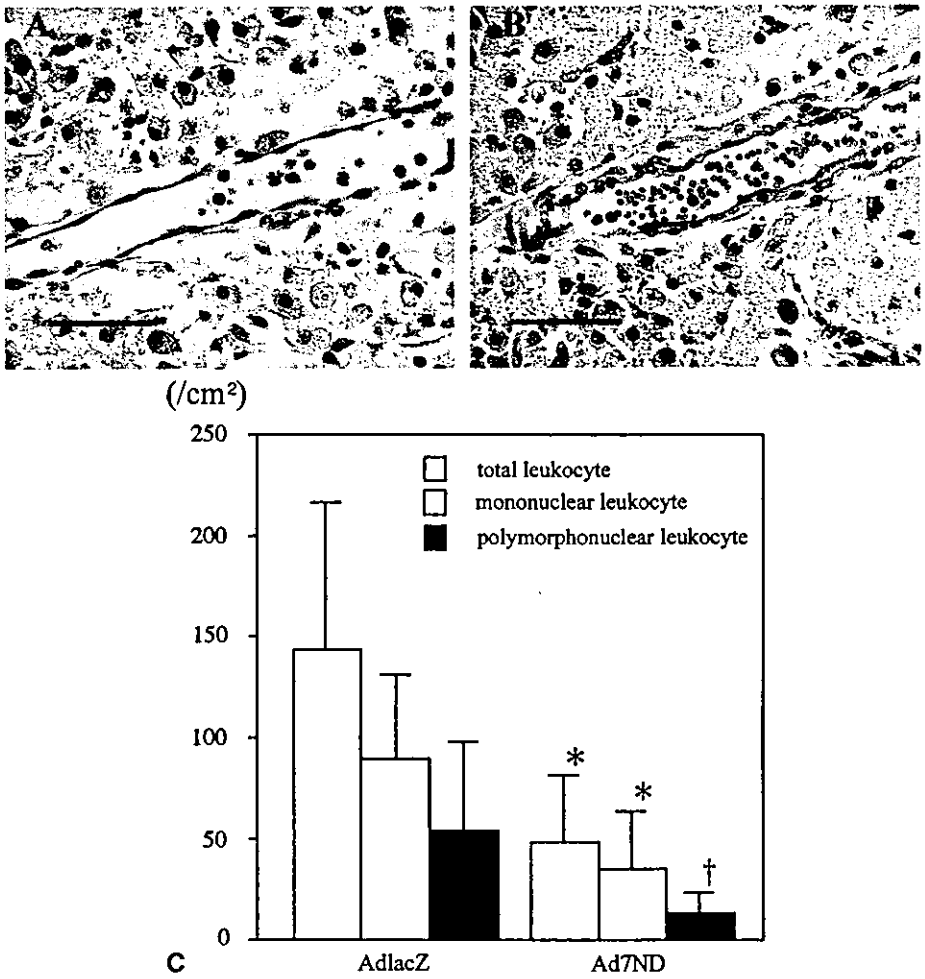

FIG.5. Leukocyte infiltration at the ischemic area after gene transfer. Hematoxylin-eosin staining of rat brains 5 days after brain ischemia and gene transfer (**A, B**; scale bar = 50 μm). Leukocyte counts in the vessels at the ischemic area were higher in the AdlacZ group (**A**) than in the Ad7ND 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.

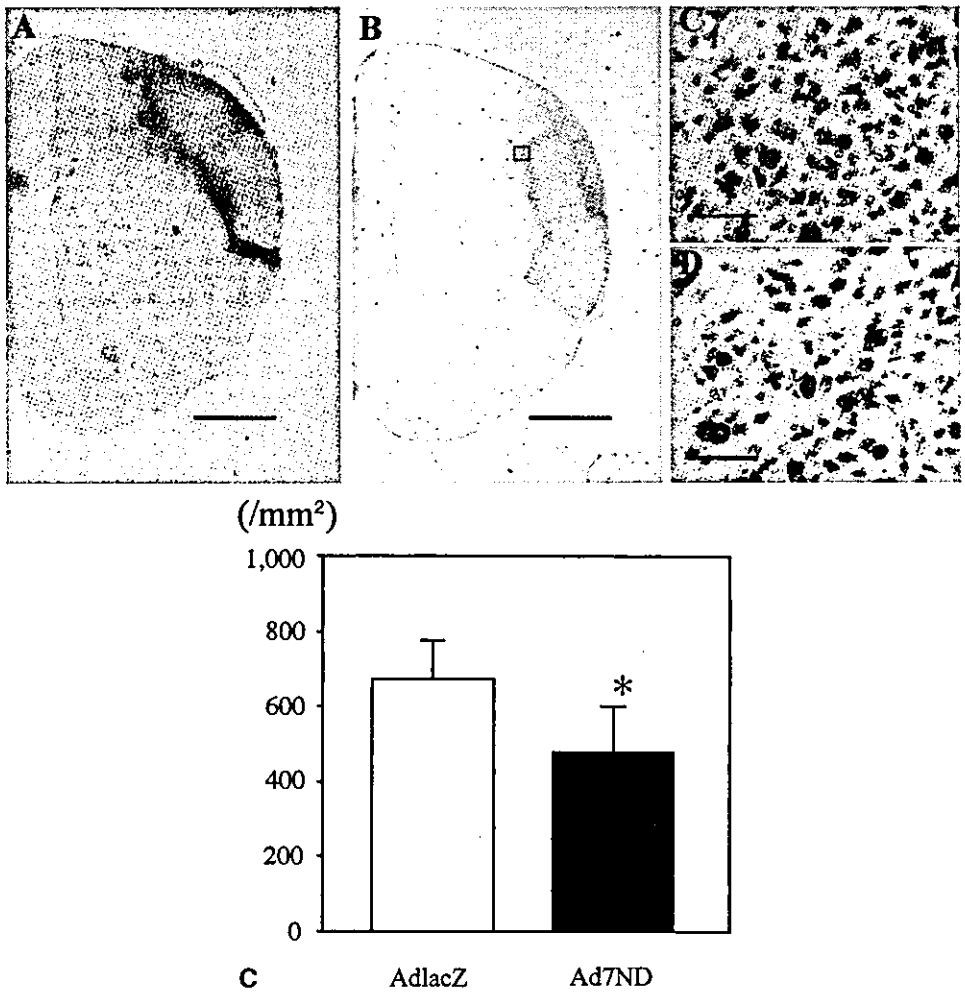

FIG. 6. Macrophage infiltration 5 days after brain ischemia and gene transfer. Sections were immunochemically stained with antimacrophage Ab, ED1 (**A–D**). Microscopic views of the brain in the AdlacZ group (**A**) or Ad7ND group (**B**) are shown in low-power fields (scale bar = 2 mm). The squares 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.

REFERENCES

Allavena P, Bianchi G, Zhou D, van Damme J, Jilek P, Sozzani S, Mantovani A (1994) Induction of natural killer cell migration by monocyte chemotactic protein-1, -2 and -3. *Eur J Immunol* 24: 3233–3236

Arvin B, Neville LF, Barone FC, Feuerstein GZ (1996) The role of inflammation and cytokines in brain injury. *Neurosci Biobehav Rev* 20:445–452

Bajocchi G, Feldman SH, Crystal RG, Mastrangeli A (1993) Direct *in vivo* gene transfer to ependymal cells in the central nervous system using recombinant adenovirus vectors. *Nat Genet* 3:229–234

Bell MD, Taub DD, Perry VH (1996) Overriding the brain's intrinsic resistance to leukocyte recruitment with intraparenchymal injections of recombinant chemokines. *Neuroscience* 74:283–292

Betz AL, Yang GY, Davidson BL (1995) Attenuation of stroke size in rats using an adenoviral vector to induce overexpression of interleukin-1 receptor antagonist in brain. *J Cereb Blood Flow Metab* 15:547–551

Boutin H, LeFeuvre RA, Horai R, Asano M, Iwakura Y, Rothwell NJ (2001) Role of IL-1α and IL-1β in ischemic brain damage. *J Neurosci* 21:5528–5534

Cai H, Yao H, Ibayashi S, Uchimura H, Fujishima M (1998) Photothrombotic middle cerebral artery occlusion in spontaneously hypertensive rats: influence of substrain, gender, and distal middle cerebral artery patterns on infarct size. *Stroke* 29:1982–1986

Carr MW, Roth SJ, Luther E, Rose SS, Springer TA (1994) Monocyte chemoattractant protein 1 acts as a T-lymphocyte chemoattractant. *Proc Natl Acad Sci U S A* 91:3652–3656

Che X, Ye W, Panga L, Wu DC, Yang GY (2001) Monocyte chemoattractant protein-1 expressed in neurons and astrocytes during focal ischemia in mice. *Brain Res* 902:171–177

Chen Y, Hallenbeck JM, Ruetzler C, Bol D, Thomas K, Berman NE, Vogel SN (2003) Overexpression of monocyte chemoattractant protein 1 in the brain exacerbates ischemic brain injury and is associated with recruitment of inflammatory cells. *J Cereb Blood Flow Metab* 23:748–755

Danton GH, Dietrich WD (2003) Inflammatory mechanisms after ischemia and stroke. *J Neuropathol Exp Neurol* 62:127–136

Dawson TC, Beck MA, Kuziel WA, Henderson F, Maeda N (2000) Contrasting effects of CCR5 and CCR2 deficiency in the pulmonary inflammatory response to influenza A virus. *Am J Pathol* 156:1951–1959

Egashira K, Koyanagi M, Kitamoto S, Ni W, Kataoka C, Morishita R, Kaneda Y, Akiyama C, Nishida KI, Sueishi K, Takeshita A (2000) Anti-monocyte chemoattractant protein-1 gene therapy inhibits vascular remodeling in rats: blockade of MCP-1 activity after intramuscular transfer of a mutant gene inhibits vascular remodeling induced by chronic blockade of NO synthesis. *FASEB J* 14:1974–1978

Egashira K (2003) Molecular mechanisms mediating inflammation in vascular disease: special reference to monocyte chemoattractant protein-1. *Hypertension* 41:834–841

Ellison JA, Barone FC, Feuerstein GZ (1999) Matrix remodeling after stroke. De novo expression of matrix proteins and integrin receptors. *Ann N Y Acad Sci* 890:204–222

Franciotta D, Martino G, Zardini E, Furlan R, Bergamaschi R, Andreoni L, Cosi V (2001) Serum and CSF levels of MCP-1 and IP-10 in multiple sclerosis patients with acute and stable disease and undergoing immunomodulatory therapies. *J Neuroimmunol* 115:192–198

Furuichi K, Wada T, Iwata Y, Kitagawa K, Kobayashi K, Hashimoto H, Ishiwata Y, Tomosugi N, Mukaida N, Matsushima K, Egashira K, Yokoyama H (2003) Gene therapy expressing amino-terminal truncated monocyte chemoattractant protein-1 prevents renal ischemia-reperfusion injury. *J Am Soc Nephrol* 14:1066–1071

Galasso JM, Liu Y, Szaflarski J, Warren JS, Silverstein FS (2000) Monocyte chemoattractant protein-1 is a mediator of acute excitotoxic injury in neonatal rat brain. *Neuroscience* 101:737–744

Hayashi K, Morishita R, Nakagami H, Yoshimura S, Hara A, Matsumoto K, Nakamura T, Ogihara T, Kaneda Y, Sakai N (2001) Gene therapy for preventing neuronal death using hepatocyte growth factor: *in vivo* gene transfer of HGF to subarachnoid space prevents delayed neuronal death in gerbil hippocampal CA1 neurons. *Gene Ther* 8:1167–1173

Hayashidani S, Tsutsui H, Shiomi T, Ikeuchi M, Matsusaka H, Suematsu N, Wen J, Egashira K, Takeshita A (2003) Anti-monocyte

chemoattractant protein-1 gene therapy attenuates left ventricular remodeling and failure after experimental myocardial infarction. *Circulation* 108:2134–2140

Heistad DD, Faraci FM (1996) Gene therapy for cerebral vascular disease. *Stroke* 27:1688–1693

Hess DC, Bhutwala T, Sheppard JC, Zhao W, Smith J (1994) ICAM-1 expression on human brain microvascular endothelial cells. *Neurosci Lett* 168:201–204

Hughes PM, Allegrini PR, Rudin M, Perry VH, Mir AK, Wiessner C (2002) Monocyte chemoattractant protein-1 deficiency is protective in a murine stroke model. *J Cereb Blood Flow Metab* 22:308–317

Hoehn B, Ringer TM, Xu L, Giffard RG, Sapolsky RM, Steinberg GK, Yenari MA (2001) Overexpression of HSP72 after induction of experimental stroke protects neurons from ischemic damage. *J Cereb Blood Flow Metab* 21:1303–1309

Ikeda Y, Yonemitsu Y, Kataoka C, Kitamoto S, Yamaoka T, Nishida K, Takeshita A, Egashira K, Sueishi K (2002) Anti-monocyte chemoattractant protein-1 gene therapy attenuates pulmonary hypertension in rats. *Am J Physiol Heart Circ Physiol* 283:H2021–H2028

Inoue S, Egashira K, Ni W, Kitamoto S, Usui M, Otani K, Ishibashi M, Hiasa K, Nishida K, Takeshita A (2002) Anti-monocyte chemoattractant protein-1 gene therapy limits progression and destabilization of established atherosclerosis in apolipoprotein E-knockout mice. *Circulation* 106:2700–2706

Jiang Y, Beller DI, Frendl G, Graves DT (1992) Monocyte chemoattractant protein-1 regulates adhesion molecule expression and cytokine production in human monocytes. *J Immunol* 148:2423–2428

Kim JS, Gautam SC, Chopp M, Zaloga C, Jones ML, Ward PA, Welch KM (1995) Expression of monocyte chemoattractant protein-1 and macrophage inflammatory protein-1 after focal cerebral ischemia in the rat. *J Neuroimmunol* 56:127–134

Koch AE, Kunkel SL, Harlow LA, Johnson B, Evanoff HL, Haines GK, Burdick MD, Pope RM, Strieter RM (1992) Enhanced production of monocyte chemoattractant protein-1 in rheumatoid arthritis. *J Clin Invest* 90:772–779

Kohara K, Tabara Y, Yamamoto Y, Igase M, Miki T (2002) *Chalmydia pneumoniae* seropositivity is associated with increased plasma levels of soluble cellular adhesion molecules in community-dwelling subjects: the Shimanami Health Promoting Program (J-SHIPP) study *Stroke* 33:1474–1479

Kumai Y, Ooboshi H, Kitazono T, Takada J, Ibayashi S, Fujishima M, Iida M (2003) Brain ischemia augments exo-focal transgene expression of adenovirus-mediated gene transfer to ependyma in hypertensive rats. *Exp Neurol* 184:904–911

Lal B, Cahan MA, Couraud PO, Goldstein GW, Laterra J (1994) Development of endogenous β-galactosidase and autofluorescence in rat brain microvessels: implications for cell tracking and gene transfer studies. *J Histochem Cytochem* 42:953–956

Lawrence MS, Sun GH, Kunis DM, Saydam TC, Dash R, Ho DY, Sapolsky, RM, Steinberg GK (1996) Overexpression of the glucose transporter gene with a herpes simplex viral vector protects striatal neurons against stroke. *J Cereb Blood Flow Metab* 16:181–185

Lawrence MS, McLaughlin JR, Sun GH, Ho DY, McIntosh L, Kunis DM, Sapolsky RM, Steinberg GK (1997) Herpes simplex viral vectors expressing Bcl-2 are neuroprotective when delivered after a stroke. *J Cereb Blood Flow Metab* 17:740–744

Linnik, MD, Zahos P, Geschwind MD, Federoff HJ (1995) Expression of bcl-2 from a defective herpes simplex virus-1 vector limits neuronal death in focal cerebral ischemia. *Stroke* 26:1670–1674

Liu TH, Beckman JS, Freeman BA, Hogan EL, Hsu CY (1989) Polyethylene glycol-conjugated superoxide dismutase and catalase reduce ischemic brain injury. *Am J Physiol* 256:H589–H593

Loddick SA, Rothwell NJ (1996) Neuroprotective effects of human recombinant interleukin-1 receptor antagonist in focal cerebral ischemia in the rat. *J Cereb Blood Flow Metab* 16:932–940

Losy J, Zaremba J (2001) Monocyte chemoattractant protein-1 is increased in the cerebrospinal fluid of patients with ischemic stroke. *Stroke* 32:2695–2696

Lu B, Rutledge BJ, Gu L, Fiorillo J, Lukacs NW, Kunkel SL, North R, Gerard C, Rollins BJ (1998) Abnormalities in monocyte recruitment and cytokine expression in monocyte chemoattractant protein 1-deficient mice. *J Exp Med* 187:601–608

Matsushima K, Larsen CG, DuBois GC, Oppenheim JJ (1989) Purification and characterization of a novel monocyte chemotactic and activating factor produced by a human myelomonocytic cell line. *J Exp Med* 169:1485–1490

Ni W, Egashira K, Kitamoto S, Kataoka C, Koyanagi M, Inoue S, Imaizumi K, Akiyama C, Nishida KI, Takeshita A (2001) New anti-monocyte chemoattractant protein-1 gene therapy attenuates atherosclerosis in apolipoprotein E-knockout mice. *Circulation* 103:2096–2101

Ni W, Kitamoto S, Ishibashi M, Usui M, Inoue S, Hiasa K, Zhao Q, Nishida K, Takeshita A, Egashira K (2004) Monocyte chemoattractant protein-1 is an essential inflammatory mediator in angiotensin II-induced progression of established atherosclerosis in hypercholesterolemic mice. *Arterioscler Thromb Vasc Biol* 24:534–539

Ooboshi H, Welsh MJ, Rios CD, Davidson BL, Heistad DD (1995) Adenovirus-mediated gene transfer *in vivo* to cerebral blood vessels and perivascular tissue. *Cir Res* 77:7–13

Ooboshi H, Rios CD, Heistad DD (1997) Novel methods for adenovirus-mediated gene transfer to blood vessels *in vivo*. *Mol Cell Biochem* 172:37–46

Ooboshi H, Ibayashi S, Takada J, Yao H, Kitazono T, Fujishima M (2001) Adenovirus-mediated gene transfer to ischemic brain. Ischemic flow threshold for transgene expression. *Stroke* 32:1043–1047

Panzer U, Stahl RA (1999) Chemokines and renal inflammation. *Nephrologie* 20:335–341

Rollins BJ (1996) Monocyte chemoattractant protein 1: a potential regulator of monocyte recruitment in inflammatory disease. *Mol Med Today* 2:198–204

Rothwell NJ, Luheshi GN (2000) Interleukin 1 in the brain: biology, pathology and therapeutic target. *Trends Neurosci* 23:618–25

Sato N, Kuziel WA, Melby PC, Reddick RL, Kostecki V, Zhao W, Maeda N, Ahuja SK, Ahuja SS (1999) Defects in the generation of IFN-gamma are overcome to control infection with Leishmania donovani in CC chemokine receptor (CCR) 5-, macrophage inflammatory protein-1 α-, or CCR2-deficient mice. *J Immunol* 163: 5519–5525

Shimazaki K, Urabe M, Monahan J, Ozawa K, Kawai N (2000) Adeno-associated virus vector-mediated bcl-2 gene transfer into post-ischemic gerbil brain in vivo: prospects for gene therapy of ischemia-induced neuronal death. *Gene Ther* 7:1244–1249

Takada J, Ooboshi H, Yao H, Kitazono T, Ibayashi S, Iida M (2003) Adenovirus-mediated gene transfer to ischemic brain is augmented in aged rats. *Exp Gerontol* 38:423–429

Takami S, Minami M, Katayama T, Nagata I, Namura S, Satoh M (2002) TAK-779, a nonpeptide CC chemokine receptor antagonist, protects the brain against focal cerebral ischemia in mice. *J Cereb Blood Flow Metab* 22:780–784

Usui M, Egashira K, Ohtani K, Kataoka C, Ishibashi M, Hiasa K, Katoh M, Zhao Q, Kitamoto S, Takeshita A (2002) Anti-monocyte chemoattractant protein-1 gene therapy inhibits restenotic changes (neointimal hyperplasia) after balloon injury in rats and monkeys. *FASEB J* 16:1838–1840

Valente AJ, Graves DT, Vialle-Valentin CE, Delgado R, Schwartz CJ (1988) Purification of a monocyte chemotactic factor secreted by nonhuman primate vascular cells in culture. *Biochemistry* 27: 4162–4168

Verma IM, Somia N (1997) Gene therapy—promises, problems and prospects. *Nature* 389;239–242

Vozzelli MA, Mason SN, Whorton MH, Auten RL Jr (2004) Anti-macrophage chemokine treatment prevents neutrophil and macrophage influx in hyperoxia-exposed newborn rat lung. *Am J Physiol Lung Cell Mol Physiol* 286:L488–493

Wang X, Yue TL, Barone FC, Feuerstein GZ (1995) Monocyte chemoattractant protein-1 messenger RNA expression in rat ischemic cortex. *Stroke* 26:661–665

Yamasaki Y, Matsuura N, Shozuhara H, Onodera H, Itoyama Y, Kogure K (1995) Interleukin-1 as a pathogenetic mediator of ischemic brain damage in rats. *Stroke* 26:676–80

Yang GY, Zhao YJ, Davidson BL, Betz AL (1997) Overexpression of

interleukin-1 receptor antagonist in the mouse brain reduces ischemic brain injury. *Brain Res* 751:181–188

Yao H, Ibayashi S, Sugimori H, Fujii K, Fujishima M (1996) Simplified model of krypton laser-induced thrombotic distal middle cerebral artery occlusion in spontaneously hypertensive rats. *Stroke* 27:333–336

Yao H, Sugimori H, Fukuda K, Takada J, Ooboshi H, Kitazono T, Ibayashi S, Iida M (2003) Photothrombotic middle cerebral artery occlusion and reperfusion laser system in spontaneously hypertensive rats. *Stroke* 34 :2716–2721

Yenari MA, Fink SL, Sun GH, Chang LK, Patel MK, Kunis DM, Onley D, Ho DY, Sapolsky RM, Steinberg GK (1998) Gene therapy with HSP72 is neuroprotective in rat models of stroke and epilepsy. *Ann Neurol* 44:584–591

Yla-Herttuala S, Lipton BA, Rosenfeld ME, Goldberg IJ, Steinberg D, Witztum JL (1991) Macrophages and smooth muscle cells express lipoprotein lipase in human and rabbit atherosclerotic lesions. *Proc Natl Acad Sci U-S A* 88:10143–10147

Yoshimura T, Robinson EA, Tanaka S, Appella E, Kuratsu J, Leonard EJ (1989) Purification and amino acid analysis of two human glioma-derived monocyte chemoattractants. *J Exp Med* 169:1449–1459

Zhang WR, Hayashi T, Iwai M, Nagano I, Sato K, Manabe Y, Abe K (2001) Time dependent amelioration against ischemic brain damage by glial cell line-derived neurotrophic factor after transient middle cerebral artery occlusion in rat. *Brain Res* 903:253–256

Zhang WR, Sato K, Iwai M, Nagano I, Manabe Y, Abe K (2002) Therapeutic time window of adenovirus-mediated GDNF gene transfer after transient middle cerebral artery occlusion in rat. *Brain Res* 947:140–145

□ CASE REPORT □

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

Kazunori TOYODA, Takeshi UWATOKO, Toshifumi SHIMADA, Noriko HAGIWARA, Shigeru FUJIMOTO, Setsuro IBAYASHI* and Yasushi OKADA

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

From the Department of Cerebrovascular Disease and Clinical Research Institute, National Kyushu Medical Center, Fukuoka and *Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka
Received for publication December 24, 2003; Accepted for publication April 27, 2004
Reprint requests should be addressed to Dr. Kazunori Toyoda, Department of Cerebrovascular Disease and Clinical Research Institute, National Kyushu Medical Center, 1-8-1 Jigyohama, Chuou-ku, Fukuoka 810-8563

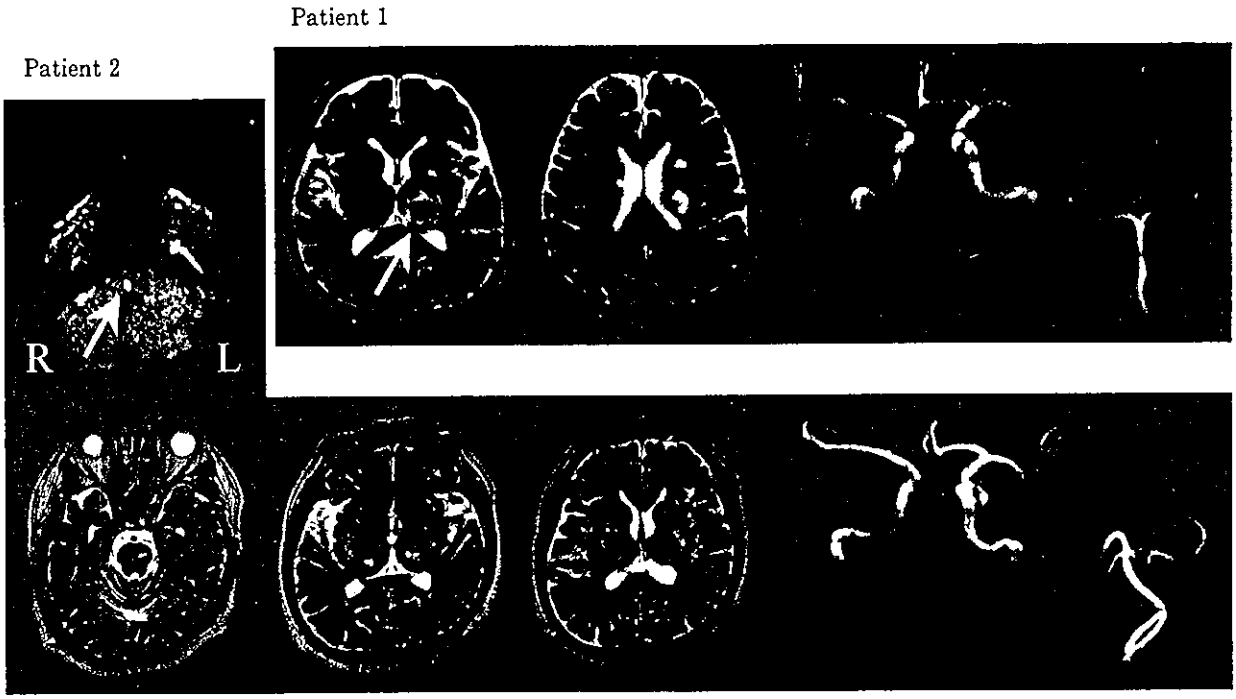

Figure 1. Cranial MRI and MRA on admission following the final stroke. T2-weighted image reveals a left thalamic hematoma in Patient 1 (arrow). Diffusion-weighted image reveals a fresh infarct in the right tegmentum of the pons in Patient 2 (arrow).

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.

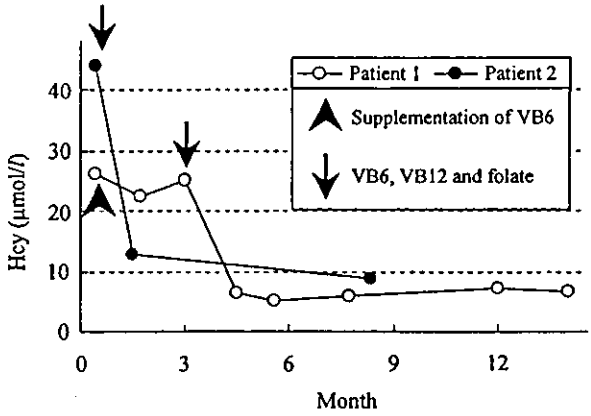

Figure 2. Changes in plasma homocysteine level following vitamin supplementation.

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.

Acknowledgements: This study was partially supported by the Japanese Ministry of Health, Labor and Welfare (15C-1).

References

1) Kelly PJ, Rosand J, Kistler JP, et al. Homocysteine, MTHFR 677C→T polymorphism, and risk of ischemic stroke: results of a meta-analysis. Neurology 59: 529–536, 2002.
2) Wald DS, Law M, Morris JK. Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis. BMJ 325: 1202, 2002.
3) Selhub J, Jacques PF, Bostom AG, et al. Association between plasma homocysteine concentrations and extracranial carotid-artery stenosis. N Engl J Med 332: 286–291, 1995.
4) Eikelboom JW, Hankey GJ, Anand SS, Lofthouse E, Staples N, Baker RI. Association between high homocyst(e)ine and ischemic stroke due to large- and small-artery disease but not other etiologic subtypes of ischemic stroke. Stroke 31: 1069–1075, 2000.
5) Sasaki T, Watanabe M, Nagai Y, et al. Association of plasma homocysteine concentration with atherosclerotic carotid plaques and lacunar infarction. Stroke 33: 1493–1496, 2002.
6) Shimizu H, Kiyohara Y, Kato I, et al. Plasma homocyst(e)ine concent-

rations and the risk of subtypes of cerebral infarction. The Hisayama study. Cerebrovasc Dis **13**: 9–15, 2002.
7) Ay H, Arsava EM, Tokgozoglu SL, Ozer N, Saribas O. Hyperhomocysteinemia is associated with the presence of left atrial thrombus in stroke patients with nonvalvular atrial fibrillation. Stroke **34**: 909–912, 2003.
8) Boysen G, Brander T, Christensen H, Gideon R, Truelsen T. Homocysteine and risk of recurrent stroke. Stroke **34**: 1258–1261, 2003.
9) Frosst P, Blom HJ, Milos R, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet **10**: 111–113, 1995.
10) Brattstrom L, Wilcken DE, Ohrvik J, Brudin L. Common methylenetetrahydrofolate reductase gene mutation leads to hyperhomocysteinemia but not to vascular disease: the result of a meta-analysis. Circulation **98**: 2520–2526, 1998.
11) Kohara K, Fujisawa M, Ando F, et al. MTHFR gene polymorphism as a risk factor for silent brain infarcts and white matter lesions in the Japanese general population: the NILS-LSA study. Stroke **34**: 1130–1135, 2003.
12) Nygard O, Vollset SE, Refsum H, et al. Total plasma homocysteine and cardiovascular risk profile. The Hordaland Homocysteine Study. JAMA **274**: 1526–1533, 1995.
13) Jacques PF, Bostom AG, Wilson PW, Rich S, Rosenberg IH, Selhub J. Determinants of plasma total homocysteine concentration in the Framingham Offspring cohort. Am J Clin Nutr **73**: 613–621, 2001.
14) Bertsch T, Mielke O, Holy S, et al. Homocysteine in cerebrovascular disease: an independent risk factor for subcortical vascular encephalopathy. Clin Chem Lab Med **39**: 721–724, 2001.
15) Vermeer SE, van Dijk EJ, Koudstaal PJ, et al. Homocysteine, silent brain infarcts, and white matter lesions: the Rotterdam Scan Study. Ann Neurol **51**: 285–289, 2002.
16) Giroud M, Lemesle M, Gouyon JB, Nivelon JL, Milan C, Dumas R. Cerebrovascular disease in children under 16 years of age in the city of Dijon, France: a study of incidence and clinical features from 1985 to 1993. J Clin Epidemiol **48**: 1343–1348, 1995.
17) Hogeveen M, Blom HJ, Van Amerongen M, Boogmans B, Van Beynum IM, Van De Bor M. Hyperhomocysteinemia as risk factor for ischemic and hemorrhagic stroke in newborn infants. J Pediatr **141**: 429–431, 2002.
18) Spence JD, Howard VJ, Chambless LE, et al. Vitamin Intervention for Stroke Prevention (VISP) trial: rationale and design. Neuroepidemiology **20**: 16–25, 2001.
19) VITATOPS Trial Study Group. The VITATOPS (Vitamins to Prevent Stroke) Trial. Rationale and design of an international, large, simple, randomised trial of homocysteine-lowering multivitamin therapy in patients with recent transient ischaemic attack or stroke. Cerebrovasc Dis **13**: 120–126, 2002.

J Neurol (2004) 251:757–759
DOI 10.1007/s00415-004-0431-2

Tetsuro Ago
Hiroaki Ooboshi
Takanari Kitazono
Tsuyoshi Imamura
Junichi Takada
Setsuro Ibayashi
Mitsuo Iida

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

Received: 23 September 2003
Received in revised form:
15 December 2003
Accepted: 13 January 2004

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

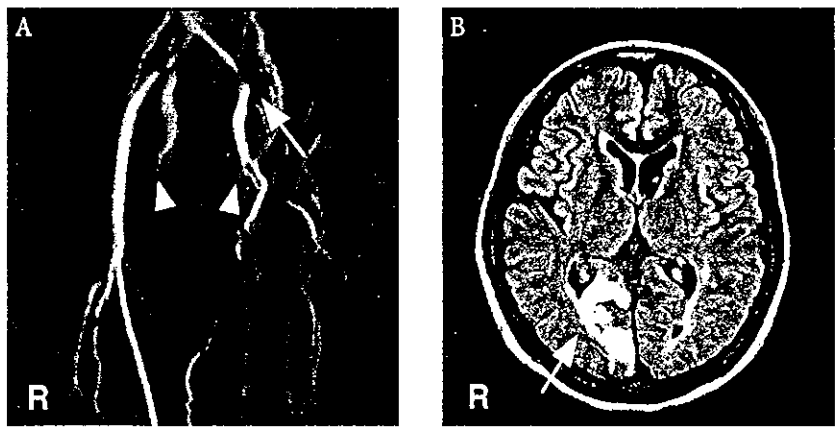

Fig. 1 Magnetic resonance imaging. **(A)** Magnetic resonance venography of lower extremities (ECG-gated two-dimensional time-of-flight) showed that bilateral femoral (arrowhead) and the left saphenous (arrow) veins were occluded with dilatation of collateral veins. **(B)** Intracranial fluid attenuated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

JON 1431

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.

References

1. Carreras LO, Vermylen JG (1982) "Lupus" anticoagulant and thrombosis – possible role of inhibition of prostacyclin formation. Thromb Haemost 48:38–40
2. Feldmann E, Levine SR (1995) Cerebrovascular disease with antiphospholipid antibodies: immune mechanisms, significance, and therapeutic options. Ann Neurol 37(Suppl 1):S114–S130
3. Fulham MJ, Gatenby P, Tuck RR (1994) Focal cerebral ischemia and antiphospholipid antibodies: a case for cardiac embolism. Acta Neurol Scand 90: 417–423
4. Galli M, Comfurius P, Maassen C, Hemker HC, de Baets MH, van Breda-Vriesman PJ, Barbui T, Zwaal RF, Bevers EM (1990) Anticardiolipin antibodies (ACA) directed not to cardiolipin but to a plasma protein cofactor. Lancet 335:1544–1547
5. Greaves M (1999) Antiphospholipid antibodies and thrombosis. LANCET 353:1348–1353
6. Khamashta MA, Cuadrado MJ, Mujic F, Taub NA, Hunt BJ, Hughes GR (1995) The management of thrombosis in the antiphospholipid-antibody syndrome. N Engl J Med 332:993–997
7. Lechat P, Mas JL, Lascault G, Loron P, Theard M, Klimczac M, Drobinski G, Thomas D, Grosgogeat Y (1988) Prevalence of patent foramen ovale in patients with stroke. N Engl J Med 318: 1148–1152
8. Lockshin MD (1992) Antiphospholipid antibody syndrome. JAMA 268: 1451–1453
9. Malia RG, Kitchen S, Greaves M, Preston FE (1990) Inhibition of activated protein C and its cofactor protein S by antiphospholipid antibodies. Br J Haematol 76:101–107
10. Martinuzzo ME, Maclouf J, Carreras LO, Levy-Toledano S (1993) Antiphospholipid antibodies enhance thrombin-induced platelet activation and thromboxane formation. Thromb Haemost 70:667–671
11. Mas JL, Arquizan C, Lamy C, Zuber M, Cabanes L, Derumeaux G, Coste J (2001) Recurrent cerebrovascular events associated with patent foramen ovale, atrial septal aneurysm, or both. N Engl J Med 345:1740–1746
12. Meister SG, Grossman W, Dexter L, Dalen JE (1972) Paradoxical embolism. Diagnosis during life. Am J Med 53: 292–298

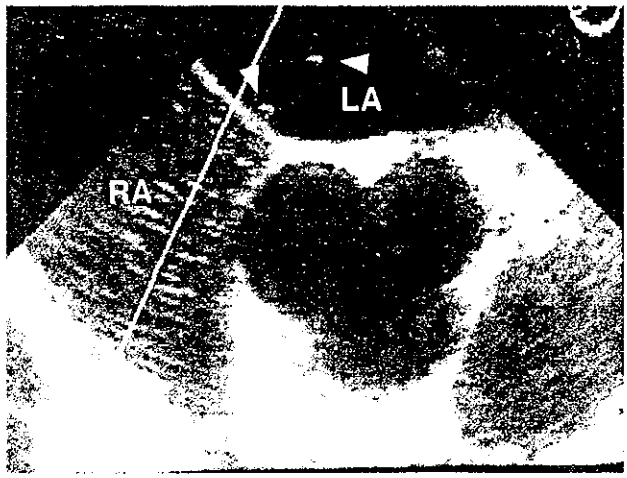

Fig. 2 Echocardiography. Transesophageal echocardiography detected microbubbles through the interatrial right-to-left shunt induced by the Valsalva-maneuver (arrowhead), indicating the presence of PFO

13. Steiner MM, Di Tullio MR, Rundek T, Gan R, Chen X, Liguori C, Brainin M, Homma S, Sacco RL (1998) Patent foramen ovale size and embolic brain imaging findings among patients with ischemic stroke. Stroke 29:944–948
14. Verro P, Levine SR, Tietjen GE (1998) Cerebrovascular ischemic events with high positive anticardiolipin antibodies. Stroke 29:2245–2253
15. Webster MW, Chancellor AM, Smith HJ, Swift DL, Sharpe DN, Bass NM, Glasgow GL (1988) Patent foramen ovale in young stroke patients. Lancet 2:11–12

Tetsuro Ago, M.D. (✉) · H. Ooboshi, MD · T. Kitazono, MD · T. Imamura, MD · J. Takada, MD · S. Ibayashi, MD · M. Iida, MD
Department of Medicine and Clinical Science
Graduate School of Medical Sciences
Kyushu University
Maidashi 3-1-1, Higashi-ku
Fukuoka 812-8582, Japan
Tel.: +81-92/642-5256
Fax: +81-92/642-5271
E-Mail: agou@intmed2.med.kyushu-u.ac.jp

ELSEVIER

Journal of the Neurological Sciences 222 (2004) 109–112

Journal of the Neurological Sciences

www.elsevier.com/locate/jns

Short communication

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

Noriko Hagiwara[a], Kazunori Toyoda[a,*], Masaru Nakayama[b], Tooru Inoue[c], Kotaro Yasumori[d], Setsuro Ibayashi[e], Yasushi Okada[a]

[a]*Department of Cerebrovascular Disease, Cerebrovascular Center and Clinical Research Institute, National Kyushu Medical Center, 1-8-1 Jigyohama, Chuou, Fukuoka 810-8563, Japan*

[b]*Department of Nephrology, Cerebrovascular Center and Clinical Research Institute, National Kyushu Medical Center, Fukuoka 810-8563, Japan*

[c]*Department of Neurosurgery, Cerebrovascular Center and Clinical Research Institute, National Kyushu Medical Center, Fukuoka 810-8563, Japan*

[d]*Department of Neuroradiology, Cerebrovascular Center and Clinical Research Institute, National Kyushu Medical Center, Fukuoka 810-8563, Japan*

[e]*Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka 812-8582, Japan*

Received 4 November 2003; received in revised form 4 March 2004; accepted 6 April 2004
Available online 24 May 2004

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.

* Corresponding author. Tel.: +81-92-852-0700; fax: +81-92-847-8802.
E-mail address: toyoda@qmed.hosp.go.jp (K. Toyoda).

0022-510X/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jns.2004.04.003

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-

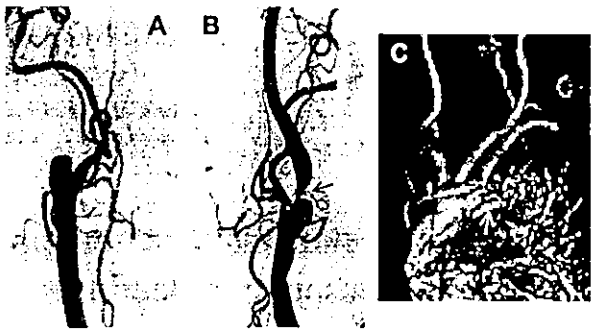

Fig. 1. Cerebrovascular images of case 1. (A) Right carotid arteriogram showed total occlusion of proximal ICA. (B) Left carotid arteriogram showed severe stenosis (73%) by NASCET's method of the proximal ICA. (C) 3D-MRA of the aortic arch showed ulcerative plaque (white arrow).

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.

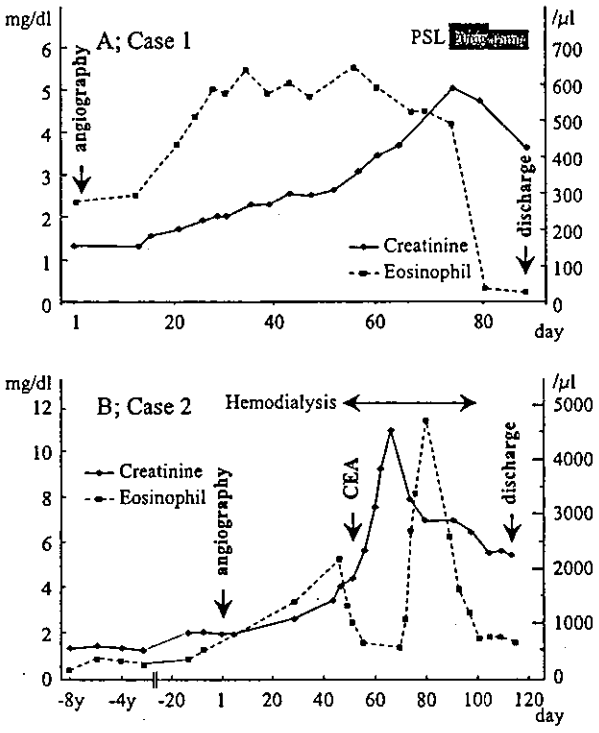

Fig. 2. Changes in serum creatinine level and eosinophil count.

2.2. Case 2

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

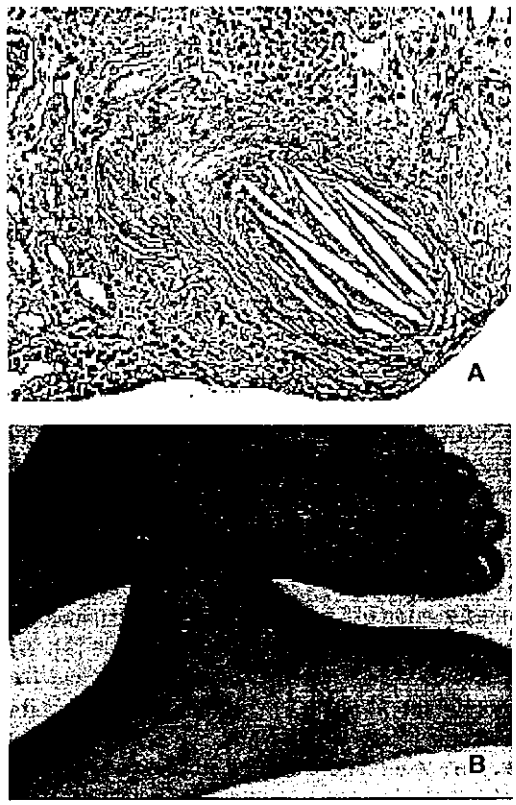

Fig. 3. Pathological findings indicative of cholesterol crystal embolism. (A) Right renal tissue specimen of patient 1 showed needle-shaped cholesterol clefts within the renal artery branch and localized neutrophilic infiltration. (B) Peripheral sites of the left foot of patient 2 showed cyanotic change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

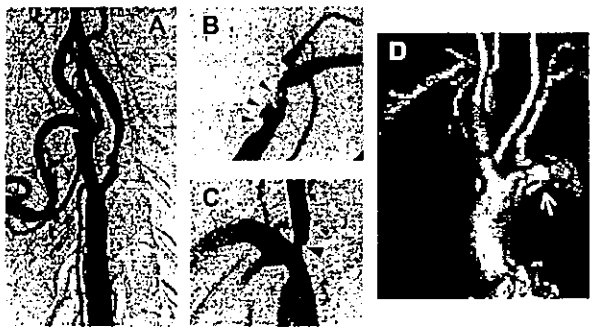

Fig. 4. Cerebrovascular images of case 2. (A) Right carotid arteriogram showed moderate stenosis (67%) by NASCET's method with ulcerative atheroma of the proximal ICA (black arrow heads). (B, C) Moderate stenosis of the left subclavian artery with ulceration (B) and right vertebral artery (C) were also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

This study was partially supported by Research Grants for Cardiovascular disease (14A-3, 15C-1) from the Ministry of Health, Labor and Welfare of Japan.

References

[1] Scolari F, Tardanico R, Zani R, et al. Cholesterol crystal embolism: a recognizable cause of renal disease. Am J Kidney Dis 2000;36: 1089–109.
[2] Balenfant Z, Meyrier A, Jacquot C. Supportive treatment improves survival in multivisceral cholesterol crystal embolism. Am J Kidney Dis 1999;33:135–8.
[3] Fine MJ, Kapoor W, Falanga V. Cholesterol crystal embolization: a review of 221 cases in the English literature. Angiology 1987;42: 769–84.
[4] Cross SS. How common is cholesterol embolism? J Clin Pathol 1991; 44:859–61.
[5] Fukumoto Y, Tsutsui H, Tsuchihashi M, Masumoto A, Takeshita A for the Cholesterol Embolism Study (CHEST) Investigators. The incidence and risk factors of cholesterol embolization syndrome, a complication of cardiac catheterization:[1]a prospective study. J Am Coll Cardiol 2003;42:211–6.
[6] Amarenco P, Duyckaerts C, Tzourio C, Henin D, Bousser MG, Hauw JJ. The prevalence of ulcerated plaques in the aortic arch in patients with stroke. N Engl J Med 1992;326:221–5.
[7] Masuda J, Yutani C, Ogata J, Kuriyama Y, Yamaguchi T. Atheromatous embolism in the brain: a Clinicopathologic analysis of 15 autopsy cases. Neurology 1994;44:1231–7.
[8] Reil T, Shekherdimian S, Golchet P, Moore W. The safety of carotid endarterectomy in patients with preoperative renal dysfunction. Ann Vasc Surg 2002;16:176–80.
[9] Flory CM. Emboli from atheromatous plaque. Am J Pathol 1945; 21:549–65.
[10] Eggebrecht H, Oldenburg O, Dirsch O, et al. Potential embolization by atherosclerotic debris dislodged from aortic wall during cardiac catheterization: histological and clinical findings in 7,621 patients. Catheter Cardiovasc Interv 2000;49:389–94.
[11] Kasinath BS, Lewis EJ. Eosinophilia as a clue to the diagnosis of Atheroembolic renal disease. Arch Intern Med 1987;147:1384–5.
[12] Fabbian F, Catalano C, Lambertini D, Bordin V, Di Landro D. A possible role of corticosteroids in cholesterol crystal embolization. Nephron 1999;83:189–90.
[13] Hasegawa M, Kawashima S, Shikano M, et al. The evaluation of corticosteroid therapy in conjunction with plasma exchange in the treatment of renal cholesterol embolic disease. A report of 5 cases. Am J Nephrol 2000;20:263–7.
[14] Woolfson RG, Lachmann H. Improvement in renal cholesterol emboli syndrome after simvastatin. Lancet 1998;351:1331–2.

Stroke December 2004; 35 2757~2758

Nicotinamide Attenuates Focal Ischemic Brain Injury: Meta-Analysis or Mechanism of Protection

To the Editor:

We read with great interest the recent article by Macleod et al.[1] They have shown a highly significant neuroprotective effect of nicotinamide on experimental stroke using the technique of systematic review or meta-analysis. The message is clear, and we believe this approach is useful and fairly robust in terms of statistical power. However, I would like to point out a possible pitfall of their overview. Nicotinamide, vitamin B3, is the precursor of nicotinamide adenine dinucleotide (NAD^+), and it may act as a poly(ADP-ribose) polymerase (PARP) inhibitor. When ischemia-induced DNA strand breakage is too extensive, the overactivation of PARP may lead to intracellular NAD^+ depletion and subsequent secondary energy failure (ie, ATP depletion).[2] ATP levels can be dramatically increased following ischemia-reperfusion by nicotinamide.[3] This scenario is generally accepted to explain the neuroprotective action of nicotinamide. However, it is also well known that high doses of nicotinamide (250 mg/kg in our case[4]) increase regional cerebral blood flow, and many studies have ignored cerebral blood flow. Therefore, we cannot jump into a conclusion that nicotinamide is a pure neuroprotectant independent of vasodilative action. If one does not consider divergent mechanisms of the beneficial effects of a candidate drug, such a promising drug based on animal experiments may fail to work under clinical settings, even though pooling of animal data reveals undoubtedly obvious neuroprotection.

Hiroshi Yao, MD
Fumiko Sadanaga-Akiyoshi, MD
Setsuro Ibayashi, MD
Mitsuo Iida, MD
Department of Medicine and Clinical Sciences
Graduate School of Medical Sciences
Fukuoka, Japan

1. Macleod MR, O'Collins T, Howells DW, Donnan GA. Pooling of animal experimental data reveals influence of study design and publication bias. *Stroke*. 2004;35:1203–1208.
2. Pieper AA, Verma A, Zhang J, Snyder SH. Poly(ADP-ribose)polymerase, nitric oxide and cell death. *Trends Pharmacol Sci*. 1999;20:171–181.
3. Klaidman L, Morales M, Kem S, Yang J, Chang M-L, Adams JD. Nicotinamide offers multiple protective mechanisms in stroke as a precursor for NAD^+, as a PARP inhibitor and by partial restoration of mitochondrial function. *Pharmacology*. 2003;69:150–157.
4. Sasanaga-Akiyoshi F, Yao H, Tanuma S, Nakahara T, Hong JS, Ibayashi S, Uchimura H, Fujishima M. Nicotinamide attenuates focal ischemic brain injury in rats: with special reference to changes in nicotinamide and NAD^+ levels in ischemic core and penumbra. *Neurochem Res*. 2003;28:1227–1234.

Response:

The comments of Yao and colleagues serve to further illustrate the potential uses of systematic review and meta-analysis in the context of the interpretation of data from animal studies in stroke.

Studies in focal cerebral ischemia may report measurement of cerebral blood flow (CBF). In most cases, they find no significant differences between control and treated groups, and use this to infer that any protective effect seen is not a consequence of changes in CBF. However, power calculations for such comparisons are seldom if ever reported; it may be that a true difference in CBF is missed because studies are underpowered for this comparison (ie, there is a type II or "false negative" error).

Analyses such as ours can illuminate the impact of study quality and study design on the estimate of efficacy. Equally, where data are available the same technique could be used to give a more precise estimate of the effect of a drug on CBF. This would reduce the probability of a type II error, and thereby reduce the risk that protection due to an effect on CBF might be incorrectly attributed to some other drug property.

It is likely that systematic review and meta-analysis can provide further insights both into the limits to the efficacy of individual drugs and into more generic determinants of outcome in experimental stroke. We are currently developing an international collaborative approach to establish priorities for future research and to develop a standard set of methodologies which might be used.

Malcolm Macleod, MRCP, PhD
Division of Molecular and Clinical Medicine
University of Edinburgh
Edinburgh, Scotland, UK

Tori O'Collins, BSci
David Howells, PhD
Geoff Donnan, MD
National Stroke Research Institute
Melbourne, Australia

原　著

卵円孔開存による奇異性脳塞栓症の発症時状況
—診断根拠としてのワルサルバ負荷・長期座位の重要性—

上床　武史[1]　豊田　一則[1]　藤本　茂[1]　矢坂　正弘[2]
井林　雪郎[3]　飯田　三雄[3]　岡田　靖[1]

要旨：若年脳卒中研究班で作成した診断基準によって奇異性脳塞栓症と診断された症例と，右左短絡を有しながら奇異性脳塞栓症と診断されなかった症例の発症時の状況に違いがみられるかについて，検討した．急性期虚血性脳血管障害をうたがわれて当施設に入院，経食道心エコーで評価をおこなった365連続例を対象とし，卵円孔開存陽性で奇異性脳塞栓症の診断にいたったA群（19例），卵円孔開存をみとめたが奇異性脳塞栓症の診断にいたらなかったB群（34例），卵円孔開存をみとめず心原性脳塞栓症と診断されたC群（69例）の3群に分けて，臨床像を比較した．A，B群をふくめて365例中78例（21%）に卵円孔開存をみとめた．発症時の状況としてワルサルバ負荷のかかる動作ないし長期の座位姿勢直後の起立をA群の37%，B群の9%，C群の3%にみとめ，A群での該当例が有意に多く（$p<0.0001$），本群に限ると静脈血栓（診断項目#3，陽性率21%）よりも頻度が高かった．奇異性脳塞栓症がワルサルバ負荷のかかる動作や長期の座位姿勢後の発症と関連することが明らかになり，その診断基準の1項目として，この特殊な発症時状況を他の項目と同等に取り扱うことも妥当と考えられた．

（臨床神経，44：503—507，2004）
Key words：脳梗塞，卵円孔開存，経食道心エコー，ワルサルバ負荷，エコノミークラス症候群

緒　言

静脈で形成された血栓が右左短絡を介して左心系に流入し，脳動脈に塞栓をおこす奇異性脳塞栓症は，若年者脳梗塞の重要な一因である[1][2]．その診断に脳梗塞の存在と右左短絡疾患の確認が必須なのはいうまでもないが，静脈血栓の同定などが診断に必要かは施設間で意見の相違があった．循環器病研究委託費12指—2若年世代の脳卒中の診断，治療，予防戦略に関する全国多施設共同研究班（Strategies against Stroke Study for Young Adults in Japan；SASSY-Japan，主任研究者：峰松一夫）では，当院をふくめた多施設後ろ向き調査結果に基づいて，Table 1に示す診断基準を作成した[3]．このうち，#6「ワルサルバ負荷のかかる動作や長期の座位姿勢での発症」に関しては，このような状況の定義を決めることが容易でなく，また後ろ向き調査で右左短絡患者における陽性率が17%と低かったため，参考所見にとどめられた．しかしながら，この低い陽性率は右左短絡を有するすべての患者を対象としたものである．偶発的に右左短絡を有していた例を外し，奇異性塞栓症をおこした可能性が高い患者群で検討すれば，#6の陽性率はより高くなる可能性がある．

本研究の目的は，Table 1の診断基準に基づいて奇異性脳塞栓症と診断された症例と，右左短絡を有しながら奇異性脳塞栓症と診断されなかった症例の臨床像，とくに発症時の状況に違いがみられるかを，検討することである．この2症例群にさらに心原性脳塞栓症と診断された症例も対比させて，奇異性脳塞栓症患者の臨床像を明確にする．なお，奇異性脳塞栓症の原因となる右左短絡には，卵円孔開存（patent foramen ovale；PFO），心房中隔欠損，心室中隔欠損，肺動静脈瘻などがあるが，本研究ではもっとも頻度が高く，かつ脳塞栓症発症に右房圧上昇の関与が強いPFOに対象を絞って，検討をおこなった．

方　法

1999年8月から2003年7月までの間に急性期虚血性脳血管障害（脳梗塞および一過性脳虚血発作）をうたがわれて当施設に入院した患者のうち，経食道心エコーで評価した365連続例を対象とした．このうち経食道心エコーでPFOをみとめ，かつTable 1の診断基準で奇異性脳塞栓症の確定（definite diagnosis）・うたがい診断（probable diagnosis）にいたったものをA群，PFOをみとめたが奇異性脳塞栓症の診断にいたらなかったものをB群，PFOをみとめず心原性脳塞栓症と診断されたものをC群に分けた．すなわちB群は，Table

[1]国立病院九州医療センター脳血管内科・臨床研究部〔〒810-8563　福岡市中央区地行浜1—8—1〕
[2]国立循環器病センター内科脳血管部門
[3]九州大学大学院医学研究院病態機能内科学
（受付日：2003年9月22日）

Table 1　Diagnostic criteria for paradoxical brain embolism[3]

# 1. Neuroradiological demonstration of brain infarction	
# 2. Presence of right-to-left shunt	
# 3. Presence of venous thrombosis	
# 4. Temporal profile or neuroradiological findings indicating embolic mechanism	
# 5. Absence of other embolic sources and stenotic lesion of major cerebral arteries	
# 6. Stroke onset following Valsalva maneuver or long-time sitting position	
Definite diagnosis	1 + 2 + 3 + 4 + 5
Probable diagnosis	1 + 2 + 3 + 4, 1 + 2 + 3 + 5, 1 + 2 + 4 + 5
Suggestive finding	6

Table 2　Clinical characteristics of the patients

Group	A n=19	B n=34	C n=69	p-value (3 groups)	p-value (A vs B)
Female gender	6 (32%)	4 (12%)	26 (38%)	< 0.05	NS
Age (y)	61 ± 14	57 ± 15	71 ± 9	< 0.0001	NS
Infarct limited at vertebro-basilar arterial territory	6 (32%)	11 (32%)	10 (15%)	NS	—
Cortical infarction	16 (84%)	22 (65%)	61 (88%)	< 0.05	NS
Multiple infarction	6 (32%)	8 (24%)	20 (29%)	NS	—
NIHSS on admission	3.6 ± 3.0	3.4 ± 4.5	7.6 ± 6.9	< 0.005	NS
mRS on discharge	1.1 ± 1.3	1.0 ± 1.3	2.1 ± 1.6	< 0.005	NS
Coagulopathy	2 (11%)	0	0	< 0.005	< 0.05
Situation at stroke onset corresponding to #6	7 (37%)	3 (9%)	2 (3%)	< 0.0001	< 0.01

1の診断基準の#1，2を満たすが，#3，4，5のうち一つ以下しか該当しなかった例を指す．経食道心エコーはHDI5000（ATL社，USA）をもちい，PFOの診断は既報にしたがいコントラスト法をもちいておこなった[3]．静脈血栓の同定には下肢静脈エコー・下肢静脈シンチグラフィー・肺血流シンチグラフィーをもちい，いずれかの検査で血栓所見をみとめれば陽性とした．3群間の臨床的特徴として，性別，年齢，脳梗塞の責任血管領域，皮質梗塞の有無，多発梗塞の有無，血液凝固異常症の有無，入院時NIH Stroke Scale（NIHSS）と退院時modified Rankin Scale（mRS），発症時の背景因子を比較検討した．発症時の背景因子として，とくに「ワルサルバ負荷のかかる動作や長期の座位姿勢での発症」（Table 1の診断項目#6）の有無をしらべた．ワルサルバ負荷に該当するか否かは，全症例の発症時状況を一覧表に無作為に記載し，他の患者情報をすべて秘匿した状態で，複数のスタッフで協議，決定した．長期座位の定義についてはSASSY-Japanの後ろ向き調査時に目安とした「60分以上の座位保持後の起立時突然発症」に加えて，30分以上の正座からの起立時突然発症も長期座位姿勢に該当するとみなした．血液凝固異常症の指標として，一般的な血栓止血学的データに加え，抗リン脂質抗体症候群の存在を示すカルジオリピン抗体，ループスアンチコアグラントや，アンチトロンビンIII，プロテインC，プロテインSを測定した．

A，B，Cの3群間での統計解析にはKruskal-Wallis検定（年齢・NIHSS・mRS）とχ^2検定（他の項目）をもちい，3群間で有意差を示した要因に関しては，Mann-WhitneyのU検定（年齢・NIHSS・mRS）とχ^2検定（他の項目）をもちいてA，B2群間の直接比較もおこなった．$p<0.05$を有意水準とした．各値を平均±標準偏差で表した．

結　果

経食道心エコーを施行した365例のうち78例（21%）にPFOをみとめ，このうち19例がA群，34例がB群に該当した．残りの25例の大半は一過性脳虚血発作の症例であった．PFO陰性の287例のうち心原性脳塞栓症の診断に該当しない脳梗塞症例や脳梗塞以外の症例を除いた69例が，C群に該当した．A群19例の全例がTable 1の診断基準の#1，2，4，5を，4例（21%）が#3を満たした．B群34例の内訳は，#1，#2，#4を満たす例が14例，#1，#2，#5を満たす