[1,2]. During this process, structural changes in extracellular matrix (ECM) were shown to play a crucial role in plaque development and disruption [3]. The structural integrity of plaques seems to depend on a balance between synthesis and degradation of the ECM which is mainly regulated by proteinases such as matrix metalloproteinases (MMPs) including interstitial collagenase or MMP-1, gelatinase A or MMP-2, stromelysin 1 or MMP-3, gelatinase B or MMP-9 [4,5].

The activities of MMPs are controlled on multiple levels: transcription and translation of their inactive precursors (zymogens), post-translational activation of zymogens by proteolysis and interactions with tissue inhibitors of metalloproteinases (TIMPs) [6] and/or tissue factor pathway inhibitor-2 (TFPI-2) [7]. Indeed, TIMPs-1, -2, -3 and TFPI-2 are expressed in atherosclerotic lesions [7–9], and these inhibitors bind to and inactivate most of the MMPs [7,10]. Thus, the expression balance of MMP to TIMP and TFPI-2 is considered to regulate the net degeneration of ECM, thus contributing to maintaining plaque stability [7,11,12]. However, few systematic data exist regarding quantitative evaluation of the expression of MMPs and their inhibitors in human atherosclerotic plaques, probably because of technical difficulties in simultaneous determination of multiple gene expression in small tissue samples obtained in clinical settings. In the present study, we used real-time reverse transcription (RT)-polymerase chain reaction (PCR) and analyzed gene expression levels of MMPs, TIMPs and TFPI-2 in human carotid plaque and an adjacent control region. We also compared expression and function of MMPs between histologically disrupted and non-disrupted plaques.

2. Subjects and methods

2.1. Subjects

The protocol of this study was approved by the institutional committee for ethical review. Written informed consent was obtained from all 24 patients who underwent carotid endarterectomy for severe stenosis of the extracranial carotid artery (all male with mean age of 68 ± 2 years). All patients presented clinical symptoms of cerebral ischemic attack related to carotid stenosis. Seven patients had a history of recent ischemic attack within 1 month prior to endarterectomy. The prevalence of risk factors for atherosclerosis was as follows: hypertension (systolic pressure >160 mmHg) in 20, hyperlipidemia (total cholesterol >220 mg/dl) in 22, smoking in 15 and diabetes mellitus (fasting blood glucose >110 mg/dl) in 10 patients. High sensitive (hs) CRP level (normal range <3 mg/l) just before surgery was 2.45 ± 0.43 mg/l (Table 1).

2.2. Tissue sampling

Samples of the plaque region were obtained immediately after endarterectomy. Endarterectomy was extended in a caudal direction to include a sample of minimally affected common carotid artery proximal to the plaque but in continuity with the plaque to act as a paired control. Under these conditions, the stenotic segment and adjacent areas were dissected undisruptedly as a single specimen, preserving circumferential integrity as much as possible. Also special care was taken not to damage luminal surface and plaque interior. After removing a part of the tissue for histological examination, all samples were immediately frozen in liquid nitrogen and stored at −80 °C until extraction of mRNA.

Procedures for RNA preparation and cDNA synthesis were already described elsewhere in detail [13]. Briefly, the samples were homogenized in 1.0 ml ISOGEN™ reagent (Nippon Gene, Tokyo, Japan), thoroughly mixed with 0.2 ml chloroform and centrifuged at 15,000 × *g* for 15 min at 4 °C. The aqueous supernatant was transferred into a micro test tube, mixed with 0.6 ml isopropanol and centrifuged at 15,000 × *g* for 15 min at 4 °C. The precipitated total RNA was rinsed with 70% ethanol, air-dried and then resuspended in RNase-free water. Then, all the total RNA was treated with DNase Free™ reagent (Ambion, Austin, TX) for 60 min, and then reverse-transcribed with Superscript II™ (Invitrogen, Carlsbad, CA) at 37 °C for 60 min using random primers (TaKaRa, Tokyo, Japan). The integrity of each cDNA mixture was checked by amplification of glutaraldehyde 3-phosphate dehydrogenase (GAPDH) with *ExTaq* (TaKaRa), using the primer set 5′-ACCACAGTCCATGCCATCAC-3′/5′-TCCACCACCCTGTTGCTGTA-3′.

Table 1
Patient Characteristics

	All patients (n = 24)	With disruption (n = 11)	Without disruption (n = 13)	p-Value
Age	68 ± 2	66 ± 3	69 ± 2	NS
Male sex	24	11	13	NS
Hypertension	20	8	12	NS
Diabetes	10	5	5	NS
HbA1c (%)	6.5 ± 0.4	7.0 ± 0.8	6.2 ± 0.4	NS
Hyperlipidemia	22	9	13	NS
LDL (mg/dl)	132 ± 6	140 ± 10	128 ± 7	NS
Smoking	15	5	10	NS
hs-CRP (mg/l)	2.45 ± 0.43	2.68 ± 0.49	2.12 ± 0.81	NS

2.3. Primers and probes for real-time RT-PCR

Using Primer Express™ software (Applied Biosystems, Foster, CA), primers were designed for each of the genes for MMP-1, -2, -3 and -9, TFPI-2 and TIMP-1, -2 and -3, and the TaqMan probe inherent to each primer set was prepared, which was an oligonucleotide labeled with a reporter dye (FAM) at the 5′-end and a quencher dye (TAMRA) at the 3′-end. The sequences of the primers and TaqMan probes of MMPs-1, -2, -3, -9, TIMPs-1, -2 and -9 were reported elsewhere [13], and those for TFPI-2 were SENSE = CGATGCTTGCTGGAGGATAGA; ANTISENSE = ACACTGGTCGTCCACACTCACT; Taqman probe = 5′-FAM-AAGTTCCCAAAGTTTGCCGGCTGC-TAMRA-3′; TFPI-2 SENSE = CGATGCTTGCTGGAGGATAGA; ANTISENSE = ACACTGGTCGTCCACACTCACT; Taqman probe = 5′-FAM-AAGTTCCCAAAGTTTGCCGGCTGC-TAMRA-3′.

Real-time RT-PCR was performed using an ABI PRISM 7700 Sequence Detection System (Applied Biosystems). The reaction solution was assembled in a volume of 25 μl, which comprised TaqMan Universal PCR Master Mix (Applied Biosystems), forward and reverse primers (final concentration 300 nM each), TaqMan probe (final concentration 200 nM) and cDNA mixture (about 2.5 ng). Throughout this study, the cDNA mixture from a particular sample was used to generate the working standard for quantitation of the cDNA of interest, which plots the relationship between the dilution of the standard cDNAs and the corresponding C_t value (the number of cycles necessary to obtain a threshold fluorescent signal) [13]. The initial quantity of the cDNA of interest in a certain cDNA mixture was calculated from the working standard and then normalized to that of GAPDH determined with TaqMan Assay Reagent Endogenous Control™ (Applied Biosystems). The normalized value for each target cDNA reflects the expression level of the corresponding gene in a test sample relative to the standard tissue. The accuracy of the present real-time RT-PCR for determining mRNA expression in human vascular tissue was already confirmed by comparing the results with those determined by conventional RT-PCR method [13].

2.4. Expression and function of MMP

To determine expression and function of MMP in its protein level, carotid tissue samples from 10 patients, in whom enough amounts of proteins could be extracted, were examined by Western blotting and gel zymography. The extracted protein was separated by SDS-PAGE and blotted onto a Hybond-ECL nitrocellulose membrane (Amersham) with the use of primary (40 μg/ml) and secondary (1:2000, Amersham) antibodies. As for zymography, proteins with gelatinolytic activity were identified by use of substrate gels prepared by incorporation of gelatin (1 mg/ml; Wako) into a SDS-PAGE. After elecrophoresis, gels were washed in 2.5% Triton X-100 for 30 min to remove SDS. The gel was equilibrated for 30 min at room temperature with gentle agitation then incubated for overnight at 37 °C in 50 mM Tris/HCl, pH 7.5, containing 0.2 M NaCl, 5 mM $CaCl_2$ and 0.02% Brij 35. Gels were then fixed and stained with 0.25% Coomassie Brilliant Blue R-250 (Wako). The product of the optical net density of the band was compared with a positive control (HT-1080 human fibrosarcoma cells for Western blotting and human MMP-2 and human MMP-9, 1.5 ng, CC073; CHEMICON for zymography) to obtain a ratio comparable between gels.

2.5. Histology and immunohistochemistry

A part of the plaque was placed in tissue fixative (Histochoice, Hedwin, Baltimore). After overnight fixation, the samples were paraffin embedded and sectioned at 4-μm intervals. Tissue sections were deparaffinized with xylene followed by immersion in graded alcohol. They were washed three times for 5 min each in phosphate-buffered saline (PBS) and blocked with bovine serum albumin for 60 min. Specimens were then incubated with primary antibodies against CD-68, MMPs, TIMPs and TFPI-2 (Fuji Chemical, Tokyo, Japan) overnight at 4 °C. After they were washed in PBS, specimens were incubated with biotinylated rabbit anti-mouse IgG for 60 min at room temperature. Specimens were then washed with PBS, stained with horseradish peroxidase-conjugated streptavidin, and finally incubated with substrate solution for 1–15 min. The tissue sections were also stained with hematoxylin–eosin and elastica van Gieson for evaluation of plaque composition and fibrous cap disruption, as described by Carr et al. [2]. Plaque was defined as atheromatous if the area of lipid core was ≥30% of the whole plaque area and as fibrous plaque if <30% in terms of its vulnerability [14].

2.6. Data analysis

The mean and standard error of triplicate data are presented. Statistical analysis was performed by paired *t*-test using Stat View 5.0 software on a Macintosh computer and by Wilcoxon matched-pair signed-rank test if appropriate. A *p*-value <0.05 was considered significant.

3. Results

3.1. Patient and plaque characteristics

Atheromatous plaque was observed in 15 samples and fibrous plaque in 9 samples. Disruption of the fibrous cap was observed in 11 samples with atheromatous plaque and was not observed in 13 samples, which consisted of 4 atheromatous and 9 fibrous plaques. Although levels of HbA1c and LDL-cholesterol in patients with plaque disruption tended to be higher than those in patients without disruption, there was no statistically significant difference in their clinical back-

Table 2
MMP, TFPI-2 and TIMP levels

Mrna	Control	Plaque	p-Value
MMP-1	0.60 ± 0.16	1.53 ± 0.25	<0.01
MMP-2	0.80 ± 0.11	0.88 ± 0.14	NS
MMP-3	0.46 ± 0.18	1.99 ± 0.59	<0.05
MMP-9	0.58 ± 0.21	2.00 ± 0.51	<0.01
TFPI-2	0.94 ± 0.23	0.32 ± 0.08	<0.01
TIMP-1	0.81 ± 0.10	1.28 ± 0.23	<0.05
TIMP-2	1.12 ± 0.15	0.95 ± 0.17	NS
TIMP-3	0.47 ± 0.16	0.67 ± 0.17	NS

ground. Also there was no difference in hs-CRP level between two patient groups, although mean value in all patients was higher than normal value (Table 1).

3.2. Expression levels of MMPs, TIMPs and TFPI-2

From removed samples with a wet weight of 11.69 ± 2.64 mg, 0.49 ± 0.22 μg total RNA was extracted for analysis. Amplification of GAPDH was equivalent among all the cDNAs synthesized. Each primer set for PCR exponentially amplified its target cDNA according to the cycle number. Normalized values for MMP, TIMP and TFPI-2 gene expression in plaque and adjacent control tissue (controls) are summarized in Table 2. In the plaques, the gene expression levels of MMP-1 (1.53 ± 0.25), MMP-3 (1.99 ± 0.59) and MMP-9 (2.00 ± 0.51) were significantly higher than those in the controls (0.60 ± 0.16, 0.46 ± 0.18 and 0.58 ± 0.21, respectively, $p < 0.05$). However, no difference was found in the expression level of MMP-2 gene between the plaques and controls (0.88 ± 0.14 versus 0.80 ± 0.11). It was quite interesting that TFPI-2 gene expression was significantly higher in the controls (0.94 ± 0.23) than that in plaques (0.32 ± 0.08, $p < 0.01$).

As for TIMP genes, the only TIMP-1 gene was significantly upregulated in plaques in comparison with that in the controls (1.28 ± 0.23 versus 0.81 ± 0.10, $p < 0.05$) (Table 2). Among the combination of the ratios of the four MMPs to the three TIMPs examined in this study, the expression ratios of MMP-1 to TIMP-1, MMP-3 to TIMP-3 and MMP-9 to TIMP-1 were significantly higher in plaques than in the controls (2.98 ± 0.77 versus 0.99 ± 0.43, 2.18 ± 0.53 versus 0.63 ± 0.22 and 1.80 ± 0.14 versus 0.83 ± 0.09, respectively, $p < 0.05$) (Fig. 1). Of interest, in plaques with disruption of fibrous cap, MMP-9 expression (3.36 ± 1.52) and the ratio of MMP-9 to TIMP-1 (1.66 ± 0.12) were significantly higher than those in plaques without disruption (1.11 ± 0.52 and 0.76 ± 0.12, respectively), although TFPI-2 gene expression was not different between these groups (0.27 ± 0.08 versus 0.40 ± 0.18).

MMP-9 protein was expressed both in disrupted and non-disrupted plaques, but was not expressed or only slightly expressed in controls. Under these conditions, net expression of MMP-9 was significantly higher in disrupted (2.61 ± 0.17,

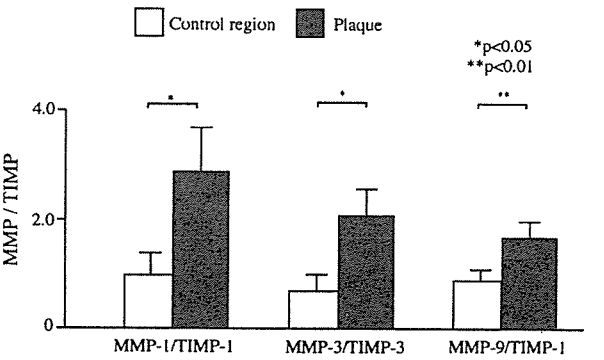

Fig. 1. Imbalanced expression of matrix metalloproteinase (MMP) to tissue inhibitor of matrix metalloproteinase (TIMP) genes in carotid plaque. Vertical axis represented the ratio of MMP/TIMP. Open columns represent values from control regions and closed columns values from plaques.

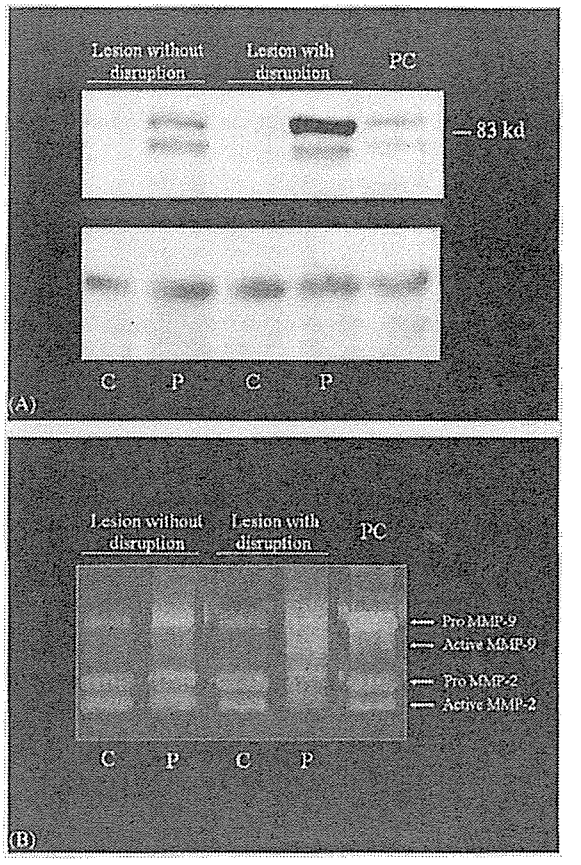

Fig. 2. Expression and function of MMP-9 in protein level. (A) Western blotting for matrix metalloproteinase MMP-9 (upper) and an internal marker protein, endothelin receptor (ETR) (lower), in non-ruptured lesion, ruptured carotid lesions and positive control (PC). MMP-9 was clearly expressed in the plaque (P) and PC, whereas in the control region (C), little expression of MMP-9 was observed. Note that both pro* and active** form of MMP-9 appears to be highly expressed in the ruptured plaque, in comparison with the non-ruptured plaque, although ETR protein is equally expressed. (B) By zymography, increased size and staining of both pro- and active forms of MMP-9 particularly in ruptured plaques, although MMP-2 activity was not different in each lane as observed in mRNA analysis.

$n=4$) than in non-disrupted plaques (1.11 ± 0.12, $n=6$, $p<0.05$), despite the equal expression of an internal marker protein, endothelin-1 receptor (Fig. 2A). Interestingly, the amount of active form MMP-9 determined by zymography was significantly higher in the disrupted (2.62 ± 0.12) than in non-disrupted plaques (0.72 ± 0.07, $p<0.05$), although pro MMP-9 activity was not significantly different in disrupted (1.8 ± 0.10) and non-disrupted plaques (1.4 ± 0.11) (Fig. 2B). There were no significant differences between the levels of pro and active forms of MMP-2, as demonstrated in its mRNA expression.

3.3. Immunohistochemistry

In the adjacent control regions (Fig. 3A), there was mild atherosclerosis where a few CD-68 positive macrophages existed. Under these conditions, MMPs and TIMPs were scatteringly positive. In contrast, TFPI-2 was diffusely positive in the intima and media. Plaque regions mainly consisted of lipid-rich core and fibrous tissue (Fig. 3B) where CD-68 positive macrophages were accumulated particularly in the shoulder regions of atheroma and all MMPs and TIMPs were strongly positive. It was interesting that, under these conditions, TFPI-2 was regionally positive in the plaque regions. Because of small number of examined plaques, we could not correlate expression of MMPs, TIMPs and TFPI-2 to the stage of plaque development.

4. Discussion

4.1. Gene expression of MMPs, TIMPs and TFPI-2 in plaque

One of the striking findings of the present study was that with a decreased TFPI-2 gene expression, the MMP-9 gene together with the MMP-9 protein was significantly upregulated in plaques, particularly in plaques with disrupted fibrous cap. Increased production of MMP-9 is thought to contribute to the progressive deterioration of the elastic lamellae associated with vessel remodeling, which could be closely related to the occurrence of plaque disruption [15]. Indeed, previous studies indicated that MMP-9 was present in the coronary plaque from unstable angina [16] and carotid plaque from

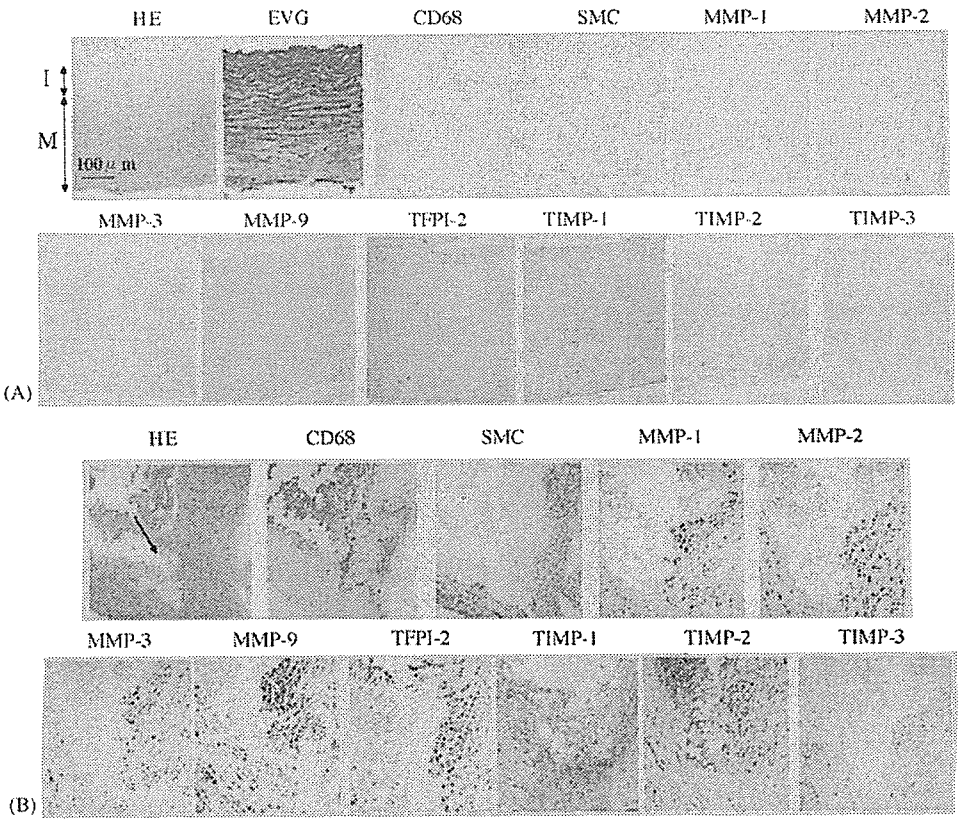

Fig. 3. Histologic and immunohistologic findings (with original magnification of ×25). (A) In the control tissues, there existed mild atherosclerotic lesion where a few CD-68 positive macrophages was found. Under these conditions, tissue factor pathway inhibitor (TFPI)-2 was diffusely positive in the intima and media, although matrix metalloproteinases (MMPs) and tissue inhibitor of MMPs (TIMPs) were scatteringly positive. An arrow indicates boundary between intima and media. (B) In plaque lesions with a lipid-rich core where CD-68 positive macrophages were accumulated, all MMPs and TIMPs were strongly positive particularly in the shoulder regions of atheroma (arrow). It was interesting that, under these conditions, TFPI-2 was regionally positive in this lesion. EVG, elastica van Gieson; HE, hematoxylin–eosin; I, intima; M, media; SMC, smooth muscle cell.

symptomatic patients [17]. We in fact demonstrated greater upregulation and function of MMP-9 in plaques with fissured fibrous cap at the mRNA as well as protein level, based on histological findings.

Simultaneous upregulation of the MMP-1 and -3 genes was also observed, as previously reported [18,19]. MMP-1 specifically cleaves collagen types I and III, which are key components of the extracellular framework of the arterial wall and major constituents of human atherosclerotic plaques, and activate other MMPs [6] that degrade denatured collagen, gelatin and elastin. MMP-3 has the widest substrate repertoire of all MMPs, showing activity against most of the extracellular proteins and proteoglycans [20]. However, unlike MMP-9, there were no differences in the expression of MMP-1 and -3 genes between plaques with and without rupture. This suggests that simultaneous upregulation of these MMPs is a plausible phenomenon in the development of atherosclerotic plaques.

This study demonstrates diminished gene expression of TFPI-2 in plaques that contain abundant MMPs. TFPI-2, originally considered as a serine proteinase inhibitor, is known to be highly expressed in smooth muscle cells of the relatively non-diseased tissue favoring ECM stability by inactivating collagenases such as MMP-1 as well as gelatinases probably through direct protein/protein interactions. Indeed, Herman et al. [7] demonstrate inverse relation between TFPI and MMP activity in atherosclerotic tissue. Thus, decreased TFPI-2 gene expression in plaques, as observed in the present study, might allow increased matrix degradation by MMP-1, -3 and -9 in plaques, enhancing their susceptibility to plaque development. It is interesting, under these conditions, TIMP-1 exhibited significantly higher expression in plaques than in controls. The combined deletion of TIMP-1 and ApoE in mice leads to a reduction in atherosclerotic plaque size [21], whereas overexpression of TIMP-1 induced by adenovirus-mediated transfer in ApoE-deficient mice leads to a decrease in plaque size and an increase in collagen content [22]. Taken together, under the condition where TFPI-2 was diminished to express, upregulation of TIMP-1 seems to counteract overexpression of MMPs, to exert an inhibitory effect on the development of atherosclerotic plaque.

However, the expression ratios of MMPs to TIMP-1 were still higher in the plaque compared with the control regions. Compensatory expression of TIMP-1 might not be sufficient to counteract the degenerative role of MMPs in the plaque, thus contributing to the development of atherosclerotic plaque. Particularly, the MMP-9/TIMP-1 ratio was significantly higher in plaques with disruption than in those without disruption. This suggests the functional significance of the imbalance of expression of these genes in the occurrence of plaque disruption. It would be of interest to examine which can play a more important role, TFPI-2 or TIMP-1, for the regulation of MMP activity, since compartmentalization might result in distinct microenvironments with corresponding variations in MMP/inhibitor ratios.

4.2. Clinical implications and limitations

A recent experimental study in which local MMP-9 was upregulated by gene transfection resulted in enhanced formation of local thrombus [23]. On the contrary, manipulation to augment expression of expression of TIMPs prevented the occurrence of plaque disruption [22]. Therefore, one might speculate that the altered balance of MMP-9/TIMP-1 with decreased TFPI-2 observed in the present study contributes to plaque disruption associated with or without regional thrombosis.

The carotid plaques examined in the present study were obtained from highly stenotic lesion probably representing the final stage of plaque development and destabilization. In acute coronary syndrome, however, atherosclerotic plaque disruption is known to occur at the sites of mild to moderate stenotic lesions [24] that were not examined in the present study. Although preliminary results indicate that in coronary plaques related to acute coronary syndrome MMP-9 gene was highly expressed in comparison with that in plaques from stable coronary disease [25], further study will need to confirm gene expression in carotid plaque from mild to moderate stenotic lesion.

The present study has a limitation regarding histological assessment of the presence of plaque disruption. Only a small portion of each plaque was examined histologically, and it may well be that features were missed in some patients. Several reports suggest that vulnerability to plaque rupture is a multifocal phenomenon particularly at the time of acute presentation [26,27]. Conversely, one might argue that we did not necessarily determine mRNA expression levels in the part of the plaque where histological analysis was performed. Even under these conditions, imbalanced expression of MMPs/TIMPs with reduction of TFPI-2 was observed in plaques, particularly in those associated with disruption. That the control regions were obtained from adjacent to the culprit lesion is another limitation. However, there was no histological evidence for plaque disruption in the control regions used for present study even in the presence of mild atherosclerosis. It can not be excluded, however, that the disruption of the fibrous cap could be resulted from surgical procedure, although we carefully examined the part of plaque where surgical procedures was not affected.

Whether upregulation of MMPs is the cause or result of plaque disarrangement is unclear. A recent study suggested that MMP-9 might have a protective effect against plaque development in double ApoE and MMP-9 knockout mice [28]. Thus, a causal relationship cannot be concluded until a controlled trial with a specific MMP-9 inhibitor is performed. Recently, MMP-8, traditionally associated only with neutrophils, which enhanced matrix breakdown by activating MMPs and/or by inactivating TIMP-1, was found to be highly expressed in macrophages in disrupted plaques [29]. Reduced expression of TFPI-2 might be related to the enhanced expression of neutrophil elastase in plaques, although MMP-

8 gene expression was not determined in the present study.

In the present study, we used real-time RT-PCR, which gives an estimate of mRNA expression instead of protein level for each enzyme and inhibitor, because it is still difficult to extract some proteases such as MMP-1, which binds strongly to connective tissue and to quantitatively assay enzyme activities [30]. However, it is important to determine the activity of TIMPs in protein level, since determination of gene expression can sometimes misinterpret the actual change of protein expression [31]. Therefore, evaluation of mRNA expression of multiple genes by the present real-time RT-PCR method in combination with determination of protein should be done for systematic evaluation of the activities of MMPs, TIMPs and TFPI-2 in clinical tissue samples.

Finally, the precise mechanism of the sustained overexpression of MMPs and TIMPs with reduction of TFPI-2 in advanced atherosclerotic plaque is still unclear. Our preliminarily report indicate that CXCR-2, a chemokine receptor, gene was highly upregulated in accordance with MMP expression in macrophages [32]. This suggests that overexpression of MMPs could be related to a continuous inflammatory reaction, although there was no difference in serum levels of hs-CRP between patients with ruptured and non-ruptured plaques. Further study of the regulatory mechanisms of chemokine and cytokine systems with transcription factors that also play a crucial role in MMP expression [33] may demonstrate a significant pathway for the expression and activation of proteinases and their inhibitors in human atherosclerotic lesions.

5. Conclusion

We applied a real-time RT-PCR method to quantitate mRNA expression in small samples of human carotid plaque. Levels of MMP-1, -3, -9 and TIMP-1 mRNAs were significantly upregulated in human carotid plaque where TFPI-2 mRNA was decreased to be expressed. The particular upregulation of MMP-9 and resultant imbalance of MMP-9/TIMP-1 expression could play a pivotal role in plaque disruption.

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Outcome of carotid endarterectomy and stent insertion based on grading of carotid endarterectomy risk: a 7-year prospective study

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Object. The authors of this study prospectively compared periprocedural neurological morbidity and the appearance of lesions on diffusion-weighted (DW) magnetic resonance (MR) imaging in patients who had undergone carotid endarterectomy (CEA) or carotid artery stent placement (CASP) with distal balloon protection, based on a CEA risk grading scale.

Methods. Patients undergoing CEA (139 patients) and CASP (92 patients) were classified into Grades I to IV, based on the presence of angiographic (Grade II), medical (Grade III), and neurological (Grade IV) risks. Although not randomized, the CEA and CASP groups were well matched in terms of the graded risk factors except for a greater proportion of neurologically unstable patients in the CEA group (11 compared with 3%, $p = 0.037$). There were greater proportions of asymptomatic (64 compared with 34%, $p = 0.006$) and North American Symptomatic Carotid Endarterectomy Trial–ineligible patients (29 compared with 14%, $p < 0.0001$) in the CASP group. The overall rates of neurological morbidity with ischemic origin and the appearance of lesions on DW MR imaging after CEA were 2.2 and 9.3%, and those after CASP were 7.6 and 35.9% (nondisabling stroke only), respectively. The only disabling stroke was caused by an intracerebral hemorrhage attributable to hyperperfusion in one case (0.7%) of CEA. There were no deaths. There was no significant association between neurological morbidity and the risk grade in patients who had undergone CEA, although the incidence of lesions on DW imaging was significantly greater in the Grade IV risk group compared with that in the other risk groups combined (42.1 compared with 4.2%, $p < 0.0001$). After CASP, a higher incidence of neurological morbidity and lesions on DW imaging was noted for the Grade II and III risk groups combined as compared with that in the Grade I risk group, regardless of a symptomatic or an asymptomatic presentation (neurological morbidity: 10.5 compared with 3.1%, respectively, $p = 0.41$; and DW imaging lesions: 47.4 compared with 19.4%, $p = 0.01$). The incidence of lesions on DW imaging after CEA was significantly lower than that after CASP except for the Grade IV risk groups.

Conclusions. Despite a higher incidence of DW imaging–demonstrated lesions in the Grade IV risk group, there was no significant association between the risk group and neurological morbidity rates after CEA. The presence of vascular and medical risk profiles conferred higher rates of neurological morbidity and an increased incidence of lesions on DW imaging after CASP. Considering that no serious nonneurological complications were noted, CEA and CASP appear to be complementary methods of revascularization for carotid artery stenosis with various risk profiles.

KEY WORDS • **carotid stenosis** • **diffusion-weighted imaging** • **endarterectomy** • **angioplasty** • **stent** • **risk factor**

CAROTID endarterectomy is the gold standard treatment for both symptomatic and asymptomatic CA stenosis.[1–4] The efficacy of this procedure in the prevention of stroke has been established in several randomized clinical trials,[1–4] and its long-term effectiveness has also been reported.[13] It is important to note, however, that this efficacy has been proven only in a subgroup of patients selected according to various criteria.[1–4] In recent years, stent implantation has been developed as an alternative to CEA for high-grade stenosis in the CAs.[37] Although its long-term efficacy in preventing stroke has not been established, CASP is, in principle, indicated in high-risk patients.[23,26,32] Nevertheless, the quest for less invasive therapeutic alternatives has led to a gradual increase in the number of CASPs performed not only in high-risk patients but also in others.[36] Several large randomized studies are now in progress to compare the safety and efficacy of CEA and CASP for the prevention of stroke in specifically defined patients.[10,15,20,38] To date, several factors have been

Abbreviations used in this paper: CA = carotid artery; CASP = CA stent placement; CCA = common CA; CEA = carotid endarterectomy; DW = diffusion-weighted; ICH = intracerebral hemorrhage; MR = magnetic resonance; NASCET = North American Symptomatic Carotid Endarterectomy Trial; TIA = transient ischemic attack.

shown to correlate with poor outcome after CASP.[7,24,28] It is important to note that classifying CA stenosis cases as high-risk for surgical intervention has been traditionally based on risks previously revealed in large CEA studies. In no previous study, however, have authors systematically compared periprocedural morbidity and cerebral embolization rates after CEA and CASP based on the CEA risk grading scale established by Sundt and colleagues.[33] In the present study we prospectively compared the neurological risks of CEA and CASP, according to this scale.

Clinical Material and Methods

Patient and Disease Characteristics

Between September 1998 and August 2004, 205 patients (187 men) with a mean age of 69.4 ± 6.7 years underwent 231 procedures for CA stenosis (139 CEAs and 92 CASPs); lesions caused symptoms in 54.1% of cases. The patients were prospectively registered, and Grades I to IV based on the CEA Risk Grading Scale were assigned.[33] Carotid endarterectomy was considered the first line of therapy; CASP was indicated mainly in high-risk patients (including those who were ineligible for NASCET), in those with echogenic plaque, and in those who expressed a preference for the procedure. Considering its extremely high surgical risk, recurrent stenosis post-CEA was a definite indication for CASP in this study. An advanced age was defined as 75 years or older.[25] The degree of CA stenosis was measured on digital subtraction angiography according to the method used in the NASCET,[17] and the indications for intervention were as follows: for symptomatic cases, 70% or greater stenosis; and for asymptomatic cases, 75% or greater stenosis. The nature of CA plaque was assessed using duplex ultrasonography. Echolucent plaques are predominantly composed of atheromatous debris, lipids, and intraplaque hemorrhage, whereas echogenic plaques mainly consist of more stable fibrous tissue.[18] Plaques with echolucent areas are more unstable and prone to fragmentation and embolization than are echogenic plaques.

Neurological Complications

The incidence of neurological complications 30 days postprocedure was calculated based on the CEA risk grade in patients assigned to either treatment group. Neurological deficits other than TIA were classified as nondisabling (that is, a new neurological deficit persisting for more than 24 hours but a modified Rankin Scale score < 3 at 30 days posttreatment) or disabling stroke (modified Rankin Scale score ≥ 3 at 30 days posttreatment).

Imaging Studies

Preoperative digital subtraction angiography was routinely performed except in one case involving an 83-year-old patient who underwent a CEA before the repair of an abdominal aortic aneurysm. The incidence of new periprocedural ischemic events, based on the CEA risk grade, was determined by comparing preoperative images with DW MR images (MAGNETOM Vision; Siemens AG, Munich, Germany) that had been obtained between 2 and 4 days after treatment. All imaging studies were performed according to the following protocol: TE 100 msec, field of view 23 × 23 cm, matrix 98 × 128, slice thickness 4 mm, and b value 1000 seconds/mm^2. When staged interventions were planned in cases of CA and coronary artery lesions, DW MR imaging studies were performed just before and after carotid revascularization to accurately assess cerebral ischemic events caused by carotid interventions.

Therapeutic Procedure

Carotid endarterectomy was performed while the patient was in a state of general anesthesia by using an operating microscope and somatosensory evoked potential monitoring to selectively place the shunt. In cases of CASP, patients had been given antiplatelet agents (aspirin 81–100 mg daily, ticlopidine 200 mg daily, cilostazol 200 mg daily, either alone or in combination) at least 48 hours before the insertion procedure, which was performed after the application of a local anesthetic agent. An intravenous heparin bolus (5000 U) was given to elevate the activated clotting time between 2- and 2.5-fold above baseline values. A No. 6-9 French guiding catheter was advanced into the CCA and placed proximal to the stenosis. A flexible guidewire (diameter 0.14 inch) or GuardWire Plus (Medtronic AVE, Minneapolis, MN) was advanced through the guiding catheter and navigated across the stenosis. The vessel was predilated with a percutaneous transluminal angioplasty balloon catheter placed across the stenosis. A balloon-expandable or self-expandable stent was then placed across the dilated segment over the guidewire by using a stent delivery system. The stents used were Palmaz (Johnson and Johnson, New Brunswick, NJ), EasyWall (Boston Scientific, Osaka, Japan), SMART or SMARTeR (Cordis, Miami Lakes, FL), Acculink (Guidant, Tokyo, Japan), Xpert (Abbott Vascular Devices, Tokyo, Japan), and others in two, 12, 72, 11, one, and two cases, respectively.

Before April 2003 (CASP I), the cerebrum was protected from distal embolism during the postdilation stage of the placement procedure by using a silicone balloon (Kaneka Medix, Kanagawa, Japan).[34] In the 18 cases treated since May 2003 (CASP II), CASP was usually performed under total protection, if possible, by using a GuardWire Plus system, temporary occlusion, and an aspiration system. Three surgeons and two interventionists in a single neurosurgical team performed the CEA and CASP procedures, respectively, during the study period.

Statistical Analysis

Differences between groups were evaluated using the chi-square or Fisher exact test (for categorical variables) and t-tests (for continuous variables). Two-sided probability values less than 0.05 were considered significant. Statistical analysis was performed using a commercially available computer software package (JMP, version 5.1.1; SAS Institute, Cary, NC).

Results

The CEA and CASP groups were similar with regard to sex and medical history despite the following differences (Table 1): the mean age of patients who had undergone CASP was significantly greater than that of those who had undergone CEA, and approximately double the number of patients who underwent CASP had had an asymptomatic presentation compared with those who underwent CEA.

TABLE 1

*Demographics of patients assigned to each treatment group**

Parameter	Value (%)		p Value
	CEA	CASP	
no. of patients	139	92	
age (yrs)			
mean ± SD	68.1 ± 6.9	71.3 ± 6.0	<0.001
range	43–82	55–83	
male sex	128 (92.0)	83 (90.2)	0.621
medical history			
DM	47 (33.8)	37 (40.2)	0.322
hyperlipidemia	15 (10.8)	16 (17.5)	0.150
hypertension	103 (74.1)	68 (73.9)	0.975
CAD	46 (33.1)	35 (38.0)	0.440
current smoker or history of smoking	71 (51.1)	40 (43.5)	0.257
PVD	65 (46.8)	41 (44.6)	0.743
target carotid stenosis, asymptomatic presentation	47 (33.8)	59 (64.1)	<0.0001
NASCET-ineligible cases			
overall	20 (14.4)	27 (29.3)	0.006
symptomatic	15 (16.3)	8 (24.2)	0.313
asymptomatic	5 (10.6)	19 (32.2)	0.008
CEA risk grade†			
I	63 (45.3)	32 (34.8)	
II	15 (10.8)	10 (10.9)	
III	42 (30.2)	47 (51.1)	
IV	19 (13.7)	3 (3.3)	

* CAD = coronary artery disease (angina pectoris or a recent myocardial infarction within 6 months); DM = diabetes mellitus; PVD= peripheral vascular disease; SD = standard deviation.

† CEA risk grades: I, neurologically stable patients with no major medical or angiographically defined risks but with unilateral or bilateral ulcerative/stenotic CA disease; II, neurologically stable patients with no major medical risks but with significant angiographically defined risks; III, neurologically stable patients with major medical risks and with or without significant angiographically defined risks; and IV, neurologically unstable patients with or without associated major medical or angiographically defined risks.

TABLE 2

*Main reasons for selecting CASP, according to the CEA risk grades**

CEA Risk Grade	Total No. of Patients	Reasons for CASP	No. of Patients
I	32	NASCET exclusion criteria	13
		restenosis post-CEA	7
		nonatheromatous stenosis	2
		cardiac valvular or rhythm disorder	2
		surgical inaccessibility	1
		previous disabling stroke	1
		asymptomatic, patient preference	12
		symptomatic, echogenic plaque	6
		symptomatic, patient preference	1
II	9	asymptomatic, patient preference	6
		NASCET exclusion criteria	2
		restenosis post-CEA	1
		tandem stenosis	1
		symptomatic, patient preference	1
III	47	advanced age (>75 yrs)	22
		NASCET exclusion criteria	9
		restenosis post-CEA	2
		age >79 yrs	1
		cardiac valvular or rhythm disorder	1
		urgent CABG	1
		recent MI	1
		surgical inaccessibility	1
		renal failure	1
		nonatheromatous stenosis	1
		angina pectoris	9
		severe PVD	6
		congestive heart failure	1
IV	3	NASCET exclusion criteria	2
		recent MI	1
		age >79 yrs	1
		advanced age (>75 yrs)	1

* The most important reason for the therapeutic choice is listed for each patient. Abbreviations: CABG = coronary artery bypass graft; MI = myocardial infarction.

Furthermore, a larger proportion of patients who had undergone CASP were NASCET-ineligible. The distribution of patients based on the grading scale by Sundt and colleagues significantly differed between the two treatment groups ($p = 0.005$): those with a Grade III risk composed approximately one half of the patients who had undergone CASP, whereas those with a Grade I risk composed approximately one half of those who had undergone CEA. Table 2 shows the main reasons for selecting CASP for each risk grade. In the Grade I risk group, CASP was indicated mainly in NASCET-ineligible cases or in patients with asymptomatic lesions who had expressed a preference for the procedure. An advanced age and NASCET exclusion criteria were the main reasons for selecting CASP in the higher risk grades.

Table 3 shows the details of the risk factors as defined according to the grading system by Sundt and colleagues. The incidence of risk profiles was similar between the two procedures, except for the greater proportion of patients who had presented with frequent TIA in the CEA group.

The overall rate of periprocedural neurological morbidity after CEA was 2.9% (disabling stroke 0.7% and nondisabling stroke 2.2%), with rates of 3.3 and 2.1% for symptomatic and asymptomatic cases, respectively. The overall rate of periprocedural neurological morbidity after CASP was 7.6% (nondisabling stroke only), with rates of 6.1 and 8.5% for symptomatic and asymptomatic cases, respectively. The incidence of ICH due to hyperperfusion syndrome was 0.4% (one case). The only disabling stroke resulted from an ICH into the previous ipsilateral cortical infarct and was caused by hyperperfusion on Day 4 after CEA for symptomatic stenosis with contralateral CA occlusion in a patient with a Grade II risk. A visual deficit caused by retinal embolism developed in two patients (2.2%) who had undergone CASP. The incidence of new DW MR imaging–demonstrated lesions in the CEA and CASP groups was 9.4 and 34.8%, respectively. All cases of periprocedural neurological morbidity, except the case of ICH, were associated with new lesions visualized on DW MR imaging, suggesting that these lesions had an ischemic origin. In both the CEA and CASP groups, symptomatic deficits were noted in approximately one fourth of the cases with new DW imaging abnormalities. Because there was a selection bias in assigning patients to the CEA and CASP groups based on the CEA risk grades, statistical analysis of the overall results was

not performed. There were no cases of acute myocardial infarction and no deaths.

Neurological Morbidity of Ischemic Origin and Cerebral Embolization Rates

The overall rate of ischemic neurological morbidity after CEA was 2.2% (nondisabling stroke only). Neurological morbidity of ischemic origin after CEA was noted in two cases with a Grade III risk and one case with a Grade IV risk. There was no significant association between the risk grade and neurological morbidity rates among patients who had undergone CEA ($p = 0.268$). Regarding DW imaging abnormalities (Table 4), there was no significant difference in the number of new lesions detected after CEA among Grades I to III risk groups ($p = 0.419$). In contrast, patients with a Grade IV risk who had undergone CEA had a higher incidence of lesions on DW imaging compared with patients in the other risk groups (42.1 compared with 4.2%, $p < 0.0001$).

All of the cerebral complications after CASP (seven cases) consisted of nondisabling ischemic stroke. Five cases were in the Grade III risk group, and one case each was in the Grades I and II risk groups (Table 4). A higher incidence of neurological deficits occurred in the CASP group among patients with angiographic or medical risks (10 and 10.6% for Grades II and III, respectively) compared with those without any risks (3.1% for Grade I; $p = 0.414$). The incidence of cerebral embolic events after CASP as detected on DW images was significantly different among the risk grades ($p = 0.028$): the incidence for risk Grades II and III combined was significantly higher than that for risk Grade I (45.6 compared with 18.8%, respectively, $p = 0.011$). Results of a subgroup analysis comparing CEA and CASP for each risk grade showed a higher incidence of lesions on imaging after CASP for Grades I ($p = 0.016$), II ($p = 0.017$), and III ($p < 0.001$) but not for Grade IV ($p = 0.273$). Despite a higher incidence of neurological morbidity after CASP for risk Grades I to III, this difference was not significant.

The introduction of the GuardWire Plus tended to decrease the overall rates of neurological complications (CASP I, 8.1%; CASP II, 5.6%; $p = 1.000$, not significant) as well as the overall rates of cerebral ischemic events on DW MR imaging (CASP I, 37.8%; CASP II, 22.2%; $p = 0.212$, not significant), although this difference did not reach statistical significance given the small number of cases. This tendency was noted chiefly in the Grade I risk group, but there seems to be virtually no effect in the Grade III risk group.

Management Outcome Based on Presentation

Results of the comparisons of periprocedural neurological morbidity and embolic event rates for both symptomatic and asymptomatic cases are listed in Table 5. Regardless of the case presentation, symptomatic or asymptomatic, vascular (Grade II) and medical (Grade III) risk profiles were associated with a higher incidence of neurological morbidity and ischemic lesions on DW MR imaging after CASP, compared with the baseline risk (Grade I). There was a significant difference in the incidence of DW imaging abnormalities between the CEA and CASP groups among Grade III risk groups with either symptomatic ($p = 0.001$) or asymptomatic presentation ($p = 0.026$).

TABLE 3

*Summary of CEA risk factors in patients assigned to CEA and CASP**

Parameter	Value (%)		p Value
	CEA	CASP	
no. of patients			
angiographic risk			
contralat ICAO	11 (7.9)	12 (13.0)	0.202
siphon stenosis	12 (8.6)	8 (8.7)	0.987
high bifurcation ($\geq C_2$)	4 (2.9)	3 (3.3)	1.000†
long lesion‡	5 (3.6)	0	0.160†
intraluminal thrombus	1 (0.7)	0	1.000†
medical risk			
advanced age (≥75 yrs)	24 (17.3)	25 (27.2)	0.071
CAD	26 (18.7)	19 (20.7)	0.715
severe PVD	7 (5.0)	9 (9.8)	0.164
severe HTN (>180/110 mm Hg)	2 (1.4)	0	0.519†
COPD	1 (0.7)	1 (1.1)	1.000†
CHF	0	1 (1.1)	0.398†
neurological risk			
frequent TIA	15 (10.8)	3 (3.3)	0.037§
general cerebral ischemia	5 (3.6)	3 (3.3)	1.000†
recent CVD (w/in 7 days)	2 (1.4)	0	0.519†

* CHF = chronic heart failure; COPD = chronic obstructive pulmonary disease; CVD = cerebrovascular disease; HTN = hypertension; ICA = internal carotid artery; ICAO = ICA occlusion.

† Fisher exact test.

‡ That is, extension of the plaque greater than 3 cm distally in the ICA or 5 cm proximally in the CCA.

§ Statistically significant.

Summary of Periprocedural Ischemic Stroke After Carotid Revascularization

Ischemic complications after CEA were caused by distal embolism during shunt insertion for high-positioned, unstable, ulcerated plaque in a symptomatic patient with cardiac comorbidity (Grade III risk) and by migration of a giant floating thrombus during the dissection phase in an asymptomatic woman with contralateral CCA occlusion (Grade III risk). The remaining complication occurred in a neurologically unstable patient who had recently had a stroke showing severe tandem siphon stenosis and moderate contralateral carotid stenosis (Grade IV risk).

Patient characteristics and presumed specific causes of stroke, if present, after CASP are listed in Table 6. The most striking features in these complicated cases, regardless of the risk grade, included the presence of severe atherosclerotic changes such as contralateral CA occlusion, bilateral severe stenosis, tandem stenosis, and cardiac comorbidities. The specific cause of stroke in a diabetic woman with cardiac comorbidity was presumed to be embolism that had occurred during placement of the guiding catheter for recently progressive CCA stenosis. Acute CA stent thrombosis was responsible for complications in two cases classified as lower risk (Grades I and II). Note that recurrent stenosis post-CEA (10 cases including one symptomatic case) was not associated with any neurological morbidity, and there was no incidence of DW imaging abnormalities.

Nonneurological complications are featured in Table 7. There was a significantly higher incidence of transient, but not permanent, cranial nerve palsy in the CEA group and a higher incidence of hypotension and bradycardia in the CASP group.

TABLE 4

Incidence of complications and DW MR imaging abnormalities after carotid revascularization, according to the CEA risk grade and surgical procedure

CEA Risk Grade	No. of Cases/Subgroup Total (%)					
	CEA	CASP	p Value	CASP I	CASP II	p Value
no. of patients	139	92		74	18	
*incidence of ischemic neurological complications**						
I	0/63 (0)	1/32 (3.1)	0.337	1/27 (3.7)	0/5 (0)	1.000
II	0/15 (0)	1/10 (10)	0.400	1/9 (16.7)	0/1 (0)	1.000
III	2/42 (4.8)	5/47 (10.6)	0.439	4/36 (11.1)	1/11 (9.1)	1.000
IV	1/19 (5.3)	0/3 (0)	1.000	0/2 (0)	0 /1 (0)	—
incidence of new abnormalities on DW MR imaging						
I	2/63 (3.2)	6/32 (18.8)	0.016	6/27 (22.2)	0/5 (0)	0.555
II	0/15 (0)	4/10 (40.0)	0.017	4/9 (44.4)	0/1 (0)	1.000
III	3/42 (7.1)	22/47 (46.8)	<0.001	18/36 (50.0)	4/11 (36.4)	0.428
IV	8/19 (42.1)	0/3 (0)	0.273	0/2 (0)	0/1 (0)	—

* All of the ischemic complications were nondisabling stroke. — = not applicable.

Discussion

This study provided a unique opportunity to compare in detail the results of CEAs and CASPs by using neurological complication rates and the incidence of lesions on DW MR imaging as outcome measures, according to the CEA risk classification. Authors of recent reports have suggested that CASP can be performed with periprocedural complication rates similar to those for CEA.[22,36] Note, however, that most studies on the safety and efficacy of CASP have included both symptomatic and asymptomatic patients and that varying definitions of stroke as clinical end points make direct comparisons of study results difficult. The grading scheme by Sundt and colleagues[33] is the only empirical system that is based on the degree of neurological stability and the presence of a set of medical and angiographic risk factors for grouping patients. It provides a means of preoperatively evaluating the risks involved in CEA,[25,33] and thus facilitates a systematic comparison of outcomes after CEA and CASP in patients with different surgical risks. We were also able to analyze these outcomes in symptomatic and asymptomatic patients separately for each risk grade, and therefore to address one of the major limitations of the system by Sundt and colleagues.

Although not randomized, the CEA and CASP groups were well matched, except for the greater number of symptomatic patients in the CEA group and NASCET-ineligible patients in the CASP group. These differences are explained by the referral patterns and our therapeutic choice of investigational CASP during the study period. Note, however, that there was no significant difference between the two treatment groups with regard to the CEA risks defined by the grading system, except for the greater number of patients with frequent TIA in the CEA group. This fact reflects an uncertainty about the superiority of either treatment based on the CEA risk profiles.

Management Outcome Based on CEA Risk

The risk of stroke and death resulting from CEA is related to a number of patient characteristics, particularly the presence and nature of recent cerebrovascular events.[9,33] The overall outcomes after CEA in the present study were excellent, and there was no significant association between risk grades and neurological morbidity after CEA. This fact may be explained by our thorough preoperative assessment of medical conditions and plaque characteristics, proper use of β-blocking agents in patients with cardiac comorbidity, and meticulous dissection under transcranial Doppler monitoring of the CA with vulnerable plaques, especially in neurologically unstable patients. The fact that the only disabling stroke was caused by an ICH attributable to hyperperfusion underscores the increasing effect of this problem, although rare, on the overall outcomes of carotid revascularization.[5,11]

The overall neurological morbidity rates and lesion incidence on DW imaging after CASP were relatively higher than those after CEA. Results of a subgroup analysis of CASP cases demonstrated higher neurological complication rates in the high-risk subset (Grades II and III). The introduction of a newer total protection system tended to decrease the overall rate of adverse neurological outcomes to 5.6%, especially in the Grade II risk group. Note, however, that neurological complication rates as well as the incidence of lesions on DW imaging after CASP for the Grade III risk group remained relatively high despite the use of advanced distal protection techniques.

Cerebral Embolic Events Detected on DW MR Imaging

Diffusion-weighted MR imaging was used to compare the incidence of ischemic lesions, given that it can detect hyperacute ischemic lesions and differentiate recent acute stroke from old ischemic lesions.[35] Results of the present study clearly showed different incidences of DW imaging abnormalities between the CEA and CASP treatment groups, based on the CEA risk grade. Not surprisingly, the incidence of bright lesions on DW MR images after CEA for cases with Grade IV risks was significantly higher than that for the other grades combined, which agrees with the hypothesis that the majority of neurological complications after CEA have an embolic origin.[16] Note, however, that the incidence of DW imaging abnormalities after CASP was relatively high—approximately 20% even in the lowest risk group (Grade I). In Grades II and III risk groups, the incidence of lesions was more than double the baseline inci-

TABLE 5

Incidence of complications and DW MR imaging abnormalities after carotid revascularization, according to the CEA risk grade, surgical procedure, and presentation

	No. of Cases/Subgroup Total (%)					
	Symptomatic			Asymptomatic		
CEA Risk Grade	CEA	CASP	p Value	CEA	CASP	p Value
incidence of ischemic neurological complications						
I	0/37 (0)	0/11 (0)	0.229	0/26 (0)	1/21 (4.8)	0.366
II	0/11 (0)	0/3 (0)	—	0/4 (0)	1/7 (14.3)	1.000
III	1/25 (4.0)	2/16 (12.5)	0.550	1/17 (5.9)	3/31 (9.7)	1.000
incidence of new abnormalities on DW MR imaging						
I	2/37 (5.4)	4/11 (36.4)	0.019	0/26 (0)	2/21 (9.5)	0.194
II	0/11 (0)	1/3 (33.3)	0.214	0/4 (0)	3/7 (42.9)	0.506
III	1/25 (4.0)	8/16 (50.0)	0.001	2/17 (11.8)	14/31 (45.2)	0.026

dence in the Grade I risk group. Because of the small number of Grade IV cases treated with CASP, the incidence rate for lesions in this group seems to be unreliable.

Authors of a recent prospective study showed that DW MR imaging detects asymptomatic bright lesions at a significant rate even after diagnostic angiography, which is a much less invasive and time-consuming procedure than CASP. The rates of lesion appearance are related to the vascular risk profile,[6] suggesting the presence of a baseline risk of embolization during catheterization of supraaortic vessels. Data from several ex vivo studies have demonstrated the risk of embolism at various stages of CA angioplasty and stent insertion, even showing an increase in the size and number of particles as the procedure proceeds from guidewire and catheter passage to angioplasty with or without stent placement.[12,29] Authors of a systematic review of the early outcome after CASP reported a reduction in thromboembolic complications during the procedure from 4.8% without protection to 0.8% with protection.[22] There was no substantial decrease in ischemic events in CASP II compared with those in CASP I, suggesting the presence of a significant risk of embolism during placement of the guiding catheter and lesion crossing by the distal balloon catheter, especially in the Grade III risk group. The risk of particulate emboli to the brain during catheterization is increased in older patients with friable atherosclerotic walls and tortuous stenosis at the carotid bifurcation. In fact, results of a previous study showed that an age of 80 or more years was the best predictor of stroke (30 days posttreatment) and death after CASP.[30] Regarding cardiac comorbidity, atherosclerosis develops earlier in the coronary arteries than in the carotid and peripheral arteries.[21] A high incidence of lesions on imaging even in the CASP II group of patients with a Grade III risk may be explained by the presence of more advanced atherosclerosis along the access route because of an advanced age and coexistent coronary and peripheral vascular risk factors. Considering the results of the study on stenting and angioplasty with protection in patients at high risk for endarterectomy (SAPPHIRE), the indications for CASP in treating NASCET-ineligible cases of high-risk CA stenosis are now expanding.[38] However, data in the present study showed that the preoperative assessment of severe atherosclerotic change along the access route, especially in the aortic arch, is mandatory when considering CASP in high-risk patients, especially those with a Grade III risk.

Results Based on Symptomatic and Asymptomatic Presentations

The findings of randomized large trials led to the creation of guidelines indicating that after CEA the expected rate of stroke 30 days posttreatment and death in patients with symptomatic or asymptomatic CA stenosis should be lower

TABLE 6

*Summary of cases with periprocedural nondisabling stroke after CASP**

Age (yrs), Sex	Presentation	CEA Risk Grade	Risk Profile/Specific Cause of Stroke
64, M	A	I	bilat severe stenosis, echolucent plaque/in-stent thrombosis, MCA thromboembolism†
69, M	A	II	contralat ICAO, tandem stenosis, misery perfusion/thrombosis at proximal edge‡
69, M	S	III	contralat ICAO, PVD, long segment CCA stenosis, echolucent plaque/dissection at distal edge, hypotension
69, M	A	III	siphon stenosis, AP, echolucent plaque, CASP before CABG
73, M	A	III	AP, SMI, echogenic calcified plaque, off-pump bypass before CASP, complicated plaque at aortic arch
74, M	A	III	AP, thoracic aortic aneurysm, CASP before total arch replacement & CABG
79, F	S	III	SMI, DM, female, PCI before CASP, progressive stenosis of CCA/placement of guiding catheter

* A = asymptomatic; AP = angina pectoris; MCA = middle cerebral artery; PCI = percutaneous coronary intervention; S = symptomatic; SMI = silent myocardial ischemia.

† Successfully treated with percutaneous transluminal angioplasty and intraarterial infusion of urokinase.

‡ Additional stenting was performed to cover the proximal edge of the primary stent.

TABLE 7
Nonneurological complications in patients assigned to each treatment group

Complication	No. (%)		p Value
	CEA	CASP	
no. of patients	139	92	
AP	3 (2.2)	2 (2.2)	1.000
congestive heart failure	0	1 (1.1)	0.398
cholesterol embolism	0	1 (1.1)	0.398
cranial nerve injury	7 (5.0)	0	0.044*
permanent	1 (0.7)	0	1.000
deep venous thrombosis	2 (1.4)	0	0.519
hypotension/bradycardia	2 (1.4)	11 (12.0)	<0.001*
pseudoaneurysm	1 (0.7)	0	1.000
respiratory distress	1 (0.7)	0	1.000
renal failure	0	1 (1.1)	0.398
wound/groin hematoma	6 (4.3)	2 (2.2)	0.482

* Statistically significant (Fisher exact test).

than 6 and 3%, respectively.[8] One of the major limitations of the grading system by Sundt and colleagues is that it does not consider the surgical risk separately for symptomatic and asymptomatic patients.[33] Furthermore, it excludes patients with recurrent stenosis post-CEA, because of the extremely high surgical risk. Results of our subgroup analysis confirmed that ischemic neurological morbidity rates and the incidence of DW imaging abnormalities after CEA and CASP were similar based on the risk grading system, regardless of the mode of presentation. Notably, the risk of CASP in asymptomatic patients with vascular and medical risk profiles is virtually the same as that in symptomatic patients and exceeds the risk recommended by the American Heart Association.[8] This finding may support our hypothesis that more advanced atherosclerosis along the access route, which is caused by an advanced patient age and coexistent vascular risk factors, may play a role in the development of embolic events in CASP; intuitively, a lesion crossing asymptomatic plaque has less potential of embolizing than one crossing symptomatic plaque. A lower profile, or smaller caliber, stent system may improve the results in such high-risk patients. Given the previously reported unfavorable results of CEA for recurrent stenosis post-CEA,[27] such cases were exclusively treated with CASP in the present study and classified into the risk grading system based on other factors. Carotid artery stent placement for recurrent stenosis post-CEA was not associated with any morbidity or DW imaging abnormalities, suggesting the benefits of CASP in cases of high surgical risk with less embolic potential, although the benefit of revascularization in asymptomatic cases remains uncertain.

Importance and Limitations of This Study

Detailed analysis of neurological ischemic complications offers important information regarding the safety of CASP in patients with different surgical risks. Authors of previous studies have reported several patient characteristics and angiographic features as high risk for CASP. Characteristics such as an advanced age of 80 or more years, uncontrolled hypertension, a recent symptomatic stroke, renal insufficiency, unforgiving hemodynamic status (neurologically unstable conditions due to misery perfusion) as well as some angiographic risk factors such as thrombus, in general, also confer increased risk for CEA. Nonetheless, some of the angiographic risk factors are unique to CASP. Tortuosity or extensive atherosclerosis of the aorta, severity of stenosis and lesion size (> 10 mm), kinks and tortuosity of the internal CA, echolucent plaque, and dense concentric calcification of the lesion confer increased risk for CASP.[14] As in these reports, most of our complicated cases had several vascular and medical risk profiles that made CASP high risk even for asymptomatic stenosis. In complicated cases in the baseline risk group (Grade I), specific causes such as acute thrombosis played a major role in assigning patients to a particular treatment group. These results showed that patients in the Grades II and III groups, considered high surgical risk groups, also had a greater risk of adverse consequences from CASP even with an asymptomatic presentation, and that meticulous periprocedural management is especially important in these patients.

Nonneurological complications after CEA and CASP deserve some mention. Myocardial infarction and cardiac dysrhythmia are the main causes of death following CEA. Notably, there was no intra- or postoperative myocardial infarction in the present study, including the NASCET-ineligible cases (20.3%). This excellent outcome regarding medical complications may be attributable to the fact that CASP was indicated mainly in patients with high medical risk profiles. Carotid endarterectomy involves an inherent risk of cranial nerve palsy although most cases are transient and mild.[16] In the present study, permanent cranial nerve palsy was noted in only one case (0.7%) treated with CEA. In contrast, CASP, especially in cases involving vascular or medical risk profiles, can cause a cholesterol embolism (the embolization of cholesterol crystals from atherosclerotic plaques of the aorta or large arteries), which may cause serious problems such as acute renal failure with a high mortality rate of 64 to 87%.[19,31]

Although the role of CASP in high-risk NASCET-ineligible patients was established especially in symptomatic cases,[38] there remains much controversy regarding its place in an unselected population with CA stenosis. Results of the present study offer insight into the mechanisms of the neurological complications in patients with various risk profiles and provide valuable information regarding patient selection for CEA and CASP based on the well-established CEA risk grading system. Although distal protection was applied during the procedure in all CASP cases in the present study, total protection using the modern distal protection device (GuardWire Plus) was performed in only one fourth (CASP II) of the cases. A preliminary comparison between CASP I and II indicated similar revascularization risk profiles based on the risk grading system, despite the overall lower complication rates in the latter group of treated cases. Nonetheless, additional studies are necessary to determine whether such revascularization risk profiles for CASP, as based on the CEA risk grading system, can be generalized to a greater number of cases also treated with total protection using the modern distal protection device.

Conclusions

Data in this study provide a unique opportunity to compare the results of CEA and CASP among patients with dif-

ferent CEA risk grades and with symptomatic or asymptomatic presentations (separately) by using neurological complication rates and the incidence of lesions on DW MR imaging as outcome measures. Despite a higher incidence of ischemic lesions in the Grade IV risk group, there was no significant association between the risk grade and neurological morbidity rates after CEA. Vascular (Grade II) and medical (Grade III) risk profiles were associated with a higher incidence of neurological morbidity and ischemic lesions on DW MR imaging after CASP, in addition to the baseline risk (Grade I), regardless of the presentation (symptomatic or asymptomatic). Recurrent stenosis post-CEA is a sound indication for CASP given the associated low incidence of neurological morbidity and DW imaging abnormalities. However, CASP should be considered carefully in asymptomatic patients with a medical risk profile. Considering that no serious nonneurological complications were noted in the present study, CEA and CASP appear to be complementary methods of revascularization for CA stenosis with various risk profiles at the present developmental stage of distal protection techniques.

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最近の大規模臨床試験の概要
外科的治療
JET study (Japanese EC-IC Bypass Trial)

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Key words：バイパス術，血行力学的脳虚血，脳循環代謝

はじめに

脳主幹動脈の慢性的な閉塞・狭窄が原因で灌流域末梢の脳血流が低下し，脳梗塞を来す血行力学的脳虚血に関しては，脳梗塞の再発予防として脳血流を術直後より増加させることの可能なバイパス術が有効であろうと考えられてきた[1-3]．

本稿では，内頸動脈あるいは中大脳動脈の慢性閉塞性病変による血行力学的脳虚血の考え方およびこれに対するバイパス術の有効性を検討したJET study (Japanese EC-IC Bypass Trial) について述べる．

1. 血行力学的脳虚血の重症度分類

脳はあらゆる臓器の中で虚血に対し，最も脆弱である．このため，脳血流を維持しようとする機構（自動調節能）が存在する．脳血管の慢性閉塞性病変により末梢の脳灌流圧が徐々に低下していくと，細動脈を拡張させ血管抵抗を低下させる．これにより脳血流は維持される．しかし，この細動脈拡張には限界があり，この限界を超えてもなお，脳灌流圧が低下すると脳血流は低下し始める．一方，少ない脳血流ながらも脳組織が正常な生命活動をするに足る酸素が何とか供給されていれば，この段階でも脳梗塞に陥らない．この酸素需要に対し酸素供給が相対的に減少している状態を貧困灌流症候群といい[4]，脳梗塞発症の準備段階と考えられている．この状態から更に，脳灌流圧が低下すると，ついに脳血流の低下により脳に対する酸素供給が絶対的に不足し脳組織が生存できなくなり，不可逆的変化，すなわち脳梗塞を来す．

2. 内頸動脈あるいは中大脳動脈の慢性閉塞性病変に対するバイパス術の考え方の変遷

バイパス術は1969年にYasargil[5]によって導入され，1970年に入って多くの施設で内頸動脈あるいは中大脳動脈の慢性閉塞性病変に対し行われるようになった．しかし，その適応および効果に関しては不明であったため，1977-82年にかけ，世界的規模で多施設参加によるprospective randomized studyが行われた[6]．そして，1985年にバイパス術は内科的治療に勝る脳梗塞再発予防効果はないとする結果が発表された[6]．この研究結果に対し，多くの批判がなされた．すなわち，多数の登録外での治療例，症例数の不足，研究期間の長期化，多数の不適合例，追跡不能例，不完全な経過観察，周術期合

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併症の多さ，脳血流からみた適応決定の曖昧さなどである．最も大きな欠点は患者選択に際し，上述した貧困灌流症候群の概念が導入されていないことにあった．すなわち，血行力学的脳虚血以外の原因で脳梗塞が再発している症例にはバイパス術は当然無効であり，また，脳主幹動脈閉塞性病変による脳梗塞の発症機序として血行力学的脳虚血は全体の10％前後と少なく，これらが，国際共同研究の結果に影響しているものと考えられた．しかし，当時貧困灌流症候群を日常臨床で診断する方法が確立しておらず，国際共同研究の結果公表後，我が国を含め慢性脳主幹動脈閉塞性病変に対するバイパス術は急速に行われなくなった．

一方，1990年代になり，positron emission tomography(PET)，single photon emission CT(SPECT)あるいはtranscranial Doppler(TCD)などの普及により脳循環代謝の測定が一般臨床でも可能となった．本来，貧困灌流は‘一定の脳血流に存在する酸素の何％が脳組織で用いられているか’の指標である酸素抽出率(oxygen extraction fraction: OEF)の上昇で表され，PETでなければ検出できないとされていた．しかし，自動調節能の出動による細動脈の拡張状態を知ることで間接的に貧困灌流を検出できることがわかってきた[7]．すなわち，血管拡張物質による脳血流の増加率が著明に減少あるいは喪失している領域は貧困灌流を来している可能性が高いことが指摘されてきた．現在，血管拡張物質として二酸化炭素あるいはacetazolamide(Diamox)が，脳血流測定装置としてはPET，SPECT，cold Xe CT，TCDが用いられている[8-12]．これらの手法を用いた多数例の脳循環代謝の測定から，①内頸動脈あるいは中大脳動脈の慢性閉塞性病変をもつ患者のうちでも，貧困灌流の存在する患者では存在しない患者に比し，有意に脳梗塞再発作を来しやすいこと[8-12]，②貧困灌流の存在する患者にバイパス術を行うと貧困灌流は消失すること[4,7]などが証明されてきた．これらの結果から，貧困灌流の存在する症例のみを集め，検討を行えばバイパス術の有効性を証明できるのではないかという気運が日本のみならず，世界的に高まってきた．

3. JET study

上述のような脳循環代謝測定法の発達およびevidence based medicine(EBM)の普及に伴い，我が国で内頸動脈あるいは中大脳動脈の慢性閉塞性病変に対するバイパス術に関してevidenceを得ようと多施設共同によるrandomized controlled trialが行われた(JET study)[13]．本研究の特徴は以下のとおりである．①脳循環の測定を定量的に高い精度で行い，貧困灌流を有する患者のみを対象とする，②対象を薬物療法のみの群あるいは薬物療法＋バイパス術の群のいずれかに無作為に割り付け，2年間追跡し，脳梗塞再発作の頻度を比較する．本研究の具体的な対象症例は[13]，内頸動脈系の閉塞性脳血管病変による一過性脳虚血発作または完成卒中を3カ月以内に認めた症例で以下のinclusion criteriaを満たすものである．すなわち，臨床的criteriaとして，73歳以下・ADLがほぼ自立している(Rankin disability scale 1，2)，放射線学的criteriaとして，CTないしはMRIにて一血管支配領域にわたる広範な脳梗塞巣を認めない・血管撮影上内頸動脈，中大脳動脈本幹の閉塞あるいは高度狭窄(CEAの対象となる内頸動脈狭窄を除く)がある，脳血流criteriaとして，3次元的定量的脳血流測定法(PET，SPECT，cold Xe CT)にて病側中大脳動脈灌流域の安静時血流量が正常値の80％未満かつacetazolamide反応性が10％未満．なお，脳血流criteriaでは登録症例を更に脳虚血の程度で中等症(0≦acetazolamide反応性＜10％)と重症(0＞acetazolamide反応性)に分けて登録した．

本研究では，1998年11月から2002年3月の3年5カ月の登録期間中に206例の登録症例があった．1998年11月1日から2002年1月31日まで(3年3カ月)の196例についての中間解析を述べる[14]．薬物療法，外科治療とも98例ずつ割り付けられていた．中等度脳虚血は104例，重度脳虚血は92例であった．primary end pointに達した症例数は，平均追跡期間15カ月で薬物療法群14例(14.3％)，外科治療群5例(5.1

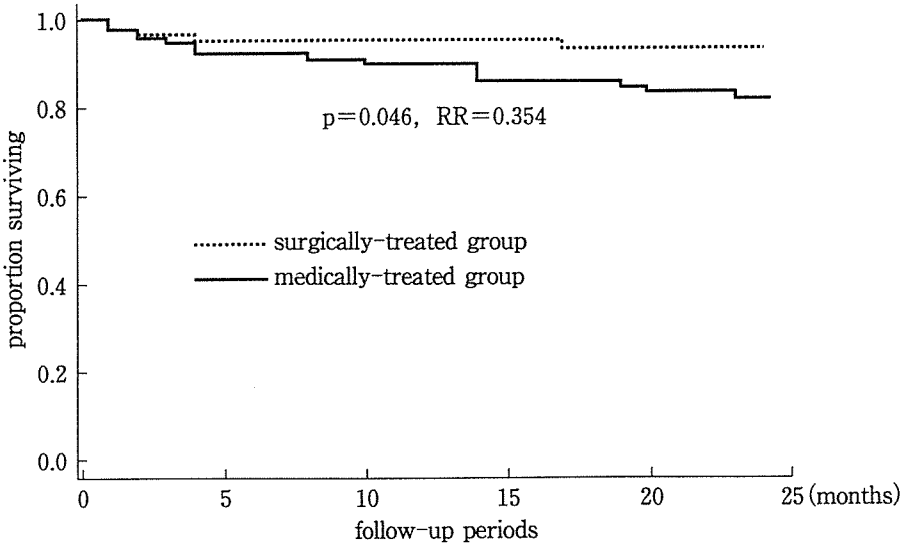

図1 JET study中間解析における primary end pointの生存曲線
薬物療法群に比し外科治療群で primary end point発生率が有意に低い.

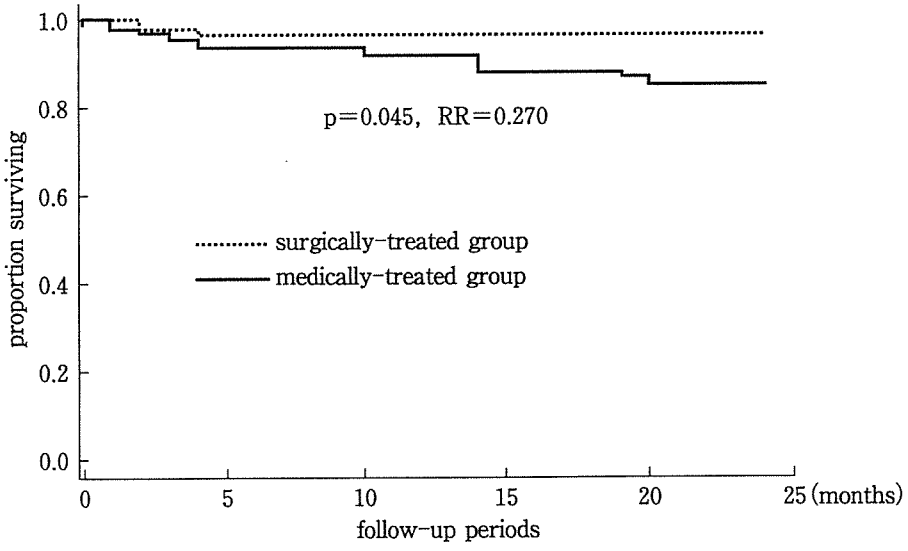

図2 JET study中間解析における secondary end pointの生存曲線
薬物療法群に比し外科治療群で secondary end point発生率が有意に低い.

%)であった. 脳虚血の重症度の比較では, 中等度脳虚血群と重度脳虚血群との間には primary end pointに達した率には有意差はなかった. Kaplan-Meier analysisによる解析では薬物療法群が外科治療群に比して有意に(p=0.046)高い頻度で primary end pointに達していた(図1). relative riskは0.354であった. primary end pointに達した症例の内訳は, 外科治療群ではプロトコール違反(登録時に既にRankin disability scale 4), 心筋梗塞による死亡, 腎不全による死亡が各1例であった. また, 同側脳梗塞再発による primary end point例が2例あった. 薬物療法群では登録時と同じ責任血管が原因の脳梗塞再発による primary end pointが11例認められた. また, 対側半球の脳梗塞および小脳脳幹部梗塞が各1例ずつ認められた. 更に, 急性心筋梗塞による primary end pointが1例認められた. secondary end pointである登録時と同側の脳梗塞再発のみについて解析すると, 薬物療法群は11.2%, 外科治療群は3.1%の頻度

でsecondary end pointに達していた．secondary end pointにおいてもやはり，脳虚血重症より中等症の方がend pointに達する頻度が高かった．Kaplan-Meier analysisによる解析では薬物療法群が外科治療群に比して有意に($p=0.045$)高い頻度でsecondary end pointに達していた(**図2**)．relative riskは0.270であった．

以上のようにJET studyの中間解析では，バイパス術に脳梗塞再発予防効果がある可能性が報告されている．我が国でのJET studyに刺激され，米国でも同様の研究方法でCOSS (Carotid Occlusion Surgery Study)が組織され，患者登録が開始されている．

おわりに

JET studyは病側中大脳動脈灌流域の安静時血流量が正常値の80％未満かつacetazolamide反応性が10％未満という重度の脳虚血のみを対象にした研究である．現在これより軽症の血行力学的脳虚血をもつ症例が薬物療法のみでどういう経過をとるかの研究をJET2 studyとして継続中である．

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