＜脳神経外科＞

①症候性右中大脳動脈狭窄症に対し、シロスタゾール(プレタール)投与により、虚血症状および狭窄の改善を認めた1例

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症例報告

症候性右中大脳動脈狭窄症に対し，シロスタゾール（プレタール）投与により，虚血症状および狭窄の改善を認めた１例

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はじめに

症候性中大脳動脈狭窄症は，内科的加療を行っても年間脳梗塞発症率が８～21%と高いとされ[1]，症候性中大脳動脈狭窄症に対する治療は，脳梗塞発症予防の上でも重要であると考えられる．今回われわれは，症候性中大脳動脈狭窄に対し，シロスタゾール（プレタール）投与により，症状の消失とともに，狭窄の改善を認めた１例を経験したので報告する．

症例提示

症　例：61歳，男性．

主　訴：左上下肢しびれ．

既　往：頸椎症．

現病歴：以前より左上肢のしびれ感が時折あり，近医にて頸椎症と診断され内服加療など受けていた．2008年９月１日より，左上肢とともに左下肢のしびれ感を自覚したため，９月２日にマツダ病院脳神経外科を受診した．初診時，意識清明で，左上下肢にしびれ感を認める以外に特記すべき神経脱落症状を認めなかった．９月５日に頭部MRIを施行したところ，拡散強調画像では明らかな急性期脳梗塞はなく，fluid attenuated inversion recovery（FLAIR）imageにて，両側の深部白質や大脳基底核に散在性の高信号域を認めるとともに，MRAにて右中大脳動脈遠位部に狭窄所見を認めた（図１）．

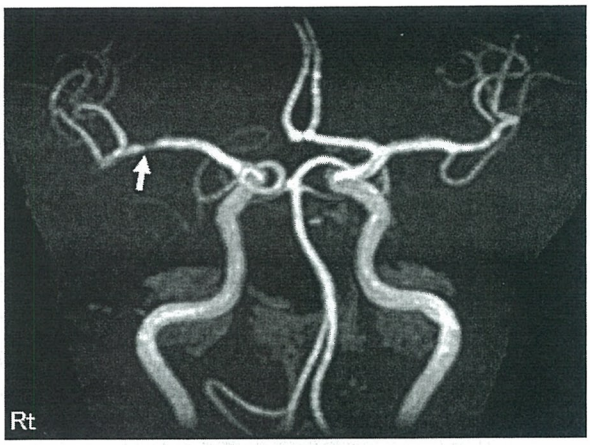

図１　初診時MRA
右中大脳動脈遠位部（矢印）に狭窄を認める．

治療経過：左上下肢のしびれと右中大脳動脈狭窄症との関連性が疑われたが，MRA上は未だ狭窄所見がそれほど高度でなく，いままで抗血小板薬を内服していなかったこともあり，シロスタゾール200 mg/day内服にて経過を観察したところ，約６カ月の経過で左上下肢のしびれは徐々に消失し，再発することもなくなった．また，2010年４月にMRI・MRAを再検査したところ，MRAにて右中大脳動脈狭窄の改善を認めた．（図２）

考　　察

感覚障害と脳虚血との関連について，Kimらは21例のpure sensory strokeを報告している[2]．主な責任病巣としては，視床や内包などの大脳基底核が挙げられて

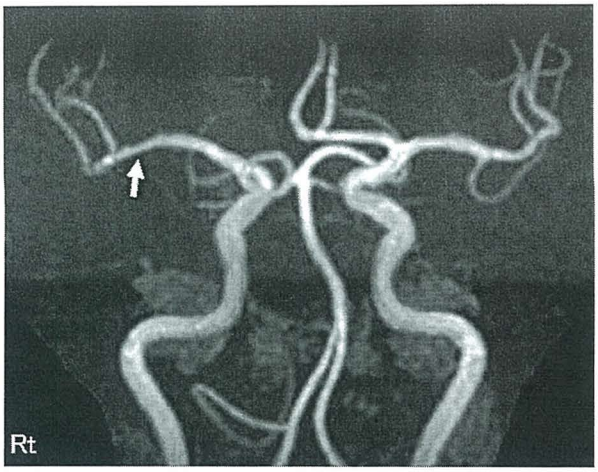

図2　1年7カ月後MRA
右中大脳動脈狭窄の改善(矢印)を認める.

いるが，その中で一過性の上肢のみの感覚障害にて発症し，頭頂葉に虚血病変を認めた1例が含まれていた．今回われわれの症例では，以前より左上肢のしびれ感があり，後に下肢にもしびれ感の拡大を認めた．さらに，シロスタゾール投与による中大脳動脈狭窄の改善とともにしびれ感も消失したことから，中大脳動脈狭窄が感覚障害と関連していた可能性は十分考えられ，また上肢のみに限局した感覚障害でも，頭蓋内病変の可能性を考慮しておく必要性があると考えられた．

症候性頭蓋内動脈狭窄病変に対する治療は，主に抗血小板療法などの保存的加療や，バルーン拡張術やステント留置術などの手術加療が挙げられる．しかしながら，現時点において保存的加療は虚血症状発生のリスクが高いとされ，ステント留置術など手術加療に期待もされているが，周術期の合併症や再狭窄の問題もある[3]．さらに，本邦においてステント留置術は保険適用になっていないのが現状であるため，症候性頭蓋内動脈狭窄に対する治療法は確立しているとはいえない状況である．

シロスタゾールはホスホジエステラーゼ3阻害薬で，細胞内のcyclic AMP濃度を増加させることにより，血小板凝集抑制作用に加えて血管内皮機能改善作用，血管拡張作用[4]，血管平滑筋細胞増殖抑制作用[5]などを示し，脳梗塞再発予防効果をもたらす．今回われわれの症例では，中大脳動脈狭窄の改善を認めたことから，シロスタゾールの血管拡張作用や血管平滑筋細胞増殖抑制作用などにより狭窄が改善したものと推測される．症候性頭蓋内動脈狭窄に対するシロスタゾールの効果を示す報告であるTOSS試験(Trial of Cilostazol in Symptomatic Intracranial Arterial Stenosis)[6]では，発症2週間以内の中大脳動脈，あるいは脳底動脈狭窄を，アスピリン治療群とアスピリン＋シロスタゾール併用群で6カ月経過観察した．狭窄病変に関してはアスピリン単独群の28.8%において進行が認められたのに対し，アスピリン＋シロスタゾール併用群では進行したのは6.7%のみで，有意に狭窄の進行が抑制されていた．症候性頭蓋内動脈狭窄に対するシロスタゾール投与は，頭痛や頻脈などの問題点もあるが，抗血小板薬としては出血性合併症の低さ[7]もあり，今後期待される治療法の1つと考えられる．

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①Plasma thrombin-cleaved osteopontin is elevation after carotid artery stenting in symptomatic ischemic stroke patients

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Plasma thrombin-cleaved osteopontin elevation after carotid artery stenting in symptomatic ischemic stroke patients

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Atherothrombosis is the primary pathophysiology that underlies ischemic cerebral infarction. Osteopontin (OPN) is produced in atherosclerotic lesions and is cleaved by activated thrombin. We hypothesized that the rupture or damage of an unstable atherosclerotic plaque increases plasma levels of thrombin-cleaved OPN (trOPN). This study included 90 patients who received carotid angioplasty with stenting (CAS), 23 patients with essential hypertension (EHT) and 10 patients who were treated with carotid endarterectomy (CEA). The CAS patient group included 36 patients that had pre- and post-operative blood tests, diffusion-weighted imaging (DWI) using cerebral MRIs and estimated thrombus debris within the protection device. Immunohistochemistry of CEA specimens revealed that trOPN was detected around intra-plaque vessels. The highest tertile of plasma trOPN levels in CAS patients was higher than trOPN levels in EHT patients. Post-operative trOPN levels were significantly higher in symptomatic compared with asymptomatic patients (P=0.003). New ipsilateral DWI-positive patients revealed higher post-operative trOPN levels (P=0.003) and a higher grade of thrombi (P<0.001) than DWI-negative patients. TrOPN may be a novel biomarker that reflects the atherothrombotic status in ischemic stroke.
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Keywords: atherosclerosis; biomarker; stenting; stroke; thrombin-cleaved osteopontin

INTRODUCTION

Lesions of the extracranial cerebral arteries, such as artery-to-artery thromboembolism, acute thrombotic occlusion resulting from plaque rupture and reduced cerebral perfusion resulting from critical stenosis or occlusion caused by progressive plaque growth, may cause ischemic stroke and transient cerebrovascular ischemia (TIA).[1] However, the thrombotic status of a patient is difficult to detect using a peripheral blood sample alone.

Osteopontin (OPN) is an extracellular matrix protein that is localized around calcified or inflammatory tissue.[2] OPN is secreted by many cell types, such as lymphocytes, macrophages, endothelial cells and vascular smooth muscle cells,[3] and exacerbates inflammation through the recruitment of monocyte macrophages and the regulation of cytokine production in macrophages, dendritic cells and T-cells.[4–6] Clinically, plasma OPN levels have a positive relationship with carotid atherosclerosis in patients with essential hypertension (EHT) and an absence of cardiovascular disease.[7] OPN expression in atherosclerotic plaques correlated with plasma OPN levels, and it was a significant predictor of future cardiovascular events in other vascular locations.[8] These data suggest that OPN is a key component in the pathophysiology of atherosclerosis.

OPN contains a thrombin cleavage site. The cleaved fragments maintain OPN adhesive function and expose new active domains that may impart new activities. The SVVYGLR cryptic domain that is exposed after thrombin cleavage induces adhesion and migration through the α4 and α9 integrins *in vitro*.[9] An antibody that neutralizes only the SLAYGLR domain of mouse OPN (homologous to the SVVYGLR of human OPN) greatly reduces the proliferation of synovial cells in a murine model of rheumatoid arthritis, which leads to bone erosion and inflammatory cell infiltration in arthritic joints.[9,10] We hypothesized that the highly thrombotic environment within atherosclerotic plaques in symptomatic stroke/TIA patients are rich in thrombin and thrombin-cleaved OPN (trOPN). TrOPN release following plaque ruptures is potentially detectable in the blood. The distribution of trOPN in atherosclerotic plaques that were removed by carotid endarterectomy was detected using immunohistochemistry to investigate this hypothesis. The plasma trOPN concentration was measured in two patient groups, asymptomatic carotid artery stenosis

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and symptomatic carotid artery stenosis, before and after carotid angioplasty with stenting (CAS), which mechanically alters plaque conformation. The relationship between plasma trOPN concentration and new ipsilateral cerebral ischemic lesions on diffusion-weighted imaging (DWI) was analyzed using magnetic resonance imaging (MRI).

METHODS

Study population

This study was a multicenter trial between Ehime University Hospital, Ehime Prefectural Imabari Hospital, Mazda Hospital (Hiroshima), Asa Citizen Hospital (Hiroshima) and Miyoshi Central Hospital (Hiroshima) from April 2008 to March 2010. The ethics committee of the Ehime University Graduate School of Medicine provided approval for the study. Informed consent was obtained from all participating patients. Patients with moderate- to high-grade carotid artery stenosis with a >70% diameter reduction who were scheduled to have CAS (n=90) or carotid endarterectomy (CEA, patients in Ehime University Hospital) were prospectively included in this study. The study design is outlined in Supplementary Figure 1. The indications for CAS or CEA were determined using CT and/or MR angiographic appearance, color duplex ultrasound, and clinical evaluation, as described previously.[11] No patients were diagnosed with acute stroke within 1 month as determined by neurological symptoms. Patients with EHT, no symptomatic cardiovascular events and no antihypertensive medications were also enrolled for comparison (Supplementary Table 1). The systolic blood pressure and diastolic blood pressure were measured in the supine position using a brachial automatic sphygmomanometer in the catheterization laboratory for CAS patients. EHT patients were measured in the seated position in the outpatient clinic. Systolic blood pressure and diastolic blood pressure were averaged using a 3-fold determination. Hypertension was defined as a systolic blood pressure over 140 mm Hg or a diastolic blood pressure over 90 mm Hg. The exclusion criteria were as follows: patients with malignant neoplasm, chronic renal failure on hemodialysis or peritoneal dialysis, autoimmune disease, or chronic inflammatory status. Patients with atrial fibrillation, a known history of cardiac thrombosis or prescribed anticoagulants were also excluded. Furthermore, all patients undergoing CAS in Ehime University Hospital were divided into two groups by pre-operative status—asymptomatic and symptomatic (Table 1). 'Symptomatic' was defined as ipsilateral cerebrovascular or ocular ischemic symptoms by a neurosurgeon before the CAS procedure within 180 days of treatment and confirmed by cerebral CT or MRI. Silent ischemic events in symptomatic patients were not included. All procedures were performed in a fasting state. Blood was collected from the peripheral veins of all CAS patients at the catheterization laboratory before the determination of OPN and trOPN levels. The same protocol was performed in all five hospitals. In Ehime University, a blood sample from peripheral veins was collected as soon as possible after the stenting procedure in 36 patients. Blood was collected from EHT patients in the morning in a fasted state after a 20-minute rest in a supine position.

Histological Examination

Plaques were divided into 5-mm-thick segments along the longitudinal axis using a standardized protocol. Segments with the greatest and smallest plaque burdens were subjected to histological examination and classified based on plaque morphology as reported previously.[12,13] Immunohistochemistry was performed on paraffin-embedded sections. Serial sections were stained with primary antibodies against macrophages (CD68, mouse monoclonal, clone PGM1, DAKO, Copenhagen, Denmark), OPN (Rabbit polyclonal, LB-4225, Cosmo Bio, Japan) and trOPN (mouse monoclonal, clone 34E3, IBL, Japan). Staining was visualized using 3,3′-diaminobenzidine tetrachloride (DAB, DAKO). Omission of the primary antibody served as specificity controls.

Carotid artery stenting

All 36 patients received antiplatelet agents. Heparin was administered (5000 IU i.v.) before stent delivery. All patients were treated with distal filter devices with a mesh size of approximately 100 μm (Angioguard Xp; Cordis). Procedures were performed under local anesthesia through percutaneous transfemoral access by three experienced interventional neurosurgeons. Ipsilateral carotid and intracranial angiography was performed to assess technical success. The amount of trapped thrombi/debris in the filters were separated into four grades after the withdrawal of protection devices: Grade 0: none, Grade 1: <20%, Grade 2: 20–50%, Grade 3: over 50%. Samples were further grouped into low grade (Grades 0 and 1) or high grade (Grades 2 and 3) (Supplementary Figure 2).

Table 1 Baseline patient characteristics in two groups, regarding pre-procedural symptom: asymptomatic and symptomatic

	Asymptomatic (n=14)	*Symptomatic (n=22)*	*P-value*
Gender (male/female)†	12/2	17/5	0.533
Age (years)	73 ± 5.7	74 ± 4.8	0.554
Current smoking, yes (%)†	8 (57.1)	16 (72.7)	0.271
Diabetes mellitus (%)†	9(64)	7(31.3)	0.058
Ischemic heart disease (%)†	7(50.0)	5(23.8)	0.109
Bilateral stenosis (%)†	5 (35.7)	11 (50.0%)	0.311
Medication			
ARB (%)†	9 (64.2)	6 (28.6)	0.040
Statin (%)†	6 (42.9)	8 (38.1)	0.526
BMI ($kg\,m^{-2}$)	23.8 ± 2.1	22.2 ± 2.3	0.046
SBP (mm Hg)	139 ± 25	140 ± 23	0.886
DBP (mm Hg)	66 ± 10	73 ± 12	0.161
Creatinine (mg per100 ml)	1.22 ± 0.23	0.88 ± 0.23	0.132
Blood glucose (mg per 100 ml)	122 ± 27	107 ± 25	0.113
HbA1c (%)	6.19 ± ±0.80	5.83 ± 0.82	0.228
HDL-C (mg per 100 ml)	58.3 ± 24.4	48.5 ± 14.5	0.168
LDL-C (mg per 100 ml)	108.1 ± 34.4	107.1 ± 37.1	0.936
Triglyceride (mg per 100 ml)	121.2 ± 54.2	133.4 ± 61.1	0.558
CRP (mg per 100 ml)††	0.18 (0.01–0.36)	0.04 (0.01–0.48)	0.170
OPN ($ng\,ml^{-1}$)	502 ± 223	599 ± 301	0.309
trOPN ($pmol\,l^{-1}$)††	0 (0–3.96)	0 (0–8.10)	0.254

Abbreviations: ARB; angiotensin II receptor blocker, BMI, body mass index; CRP, c reactive protein; DBP, diastolic blood pressure; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; OPN, osteopontin; SBP, systolic blood pressure; trOPN, thrombin-cleaved form of osteopontin.
Statistical analysis was performed by Student's t test: †χ^2-test; ††Mann–Whitney's U test.

OPN enzyme-linked immunosorbent assay

Blood samples were centrifuged and stored at −80 °C until measurement. Plasma OPN and trOPN levels were measured using a commercial enzyme-linked immunosorbent assay kit (Immuno Biological Laboratory, Gunma, Japan, code 27156 and 27258, respectively) according to the described procedures. All measurements were performed in duplicate.

Radiological assessment

Carotid stenosis was classified using CT angiography according to consensus criteria.[14] DWI was performed within 7 days before and 2 days after the CAS procedure, and assessed by experienced radiologists who were blinded to all of the clinical details.

Statistical analysis

Comparisons of two groups, such as symptomatic or asymptomatic and EHT or CAS patients, were analyzed using the Student's t test, χ^2-test, or Mann–Whitney U test. The relationship between OPN, trOPN and other parameters in CAS patients were analyzed using the Pearson's correlation test or Spearman's rank-order test. The paired t test and Wilcoxon test analyzed changes in OPN and trOPN levels, respectively. A P value of <0.05 was considered statistically significant.

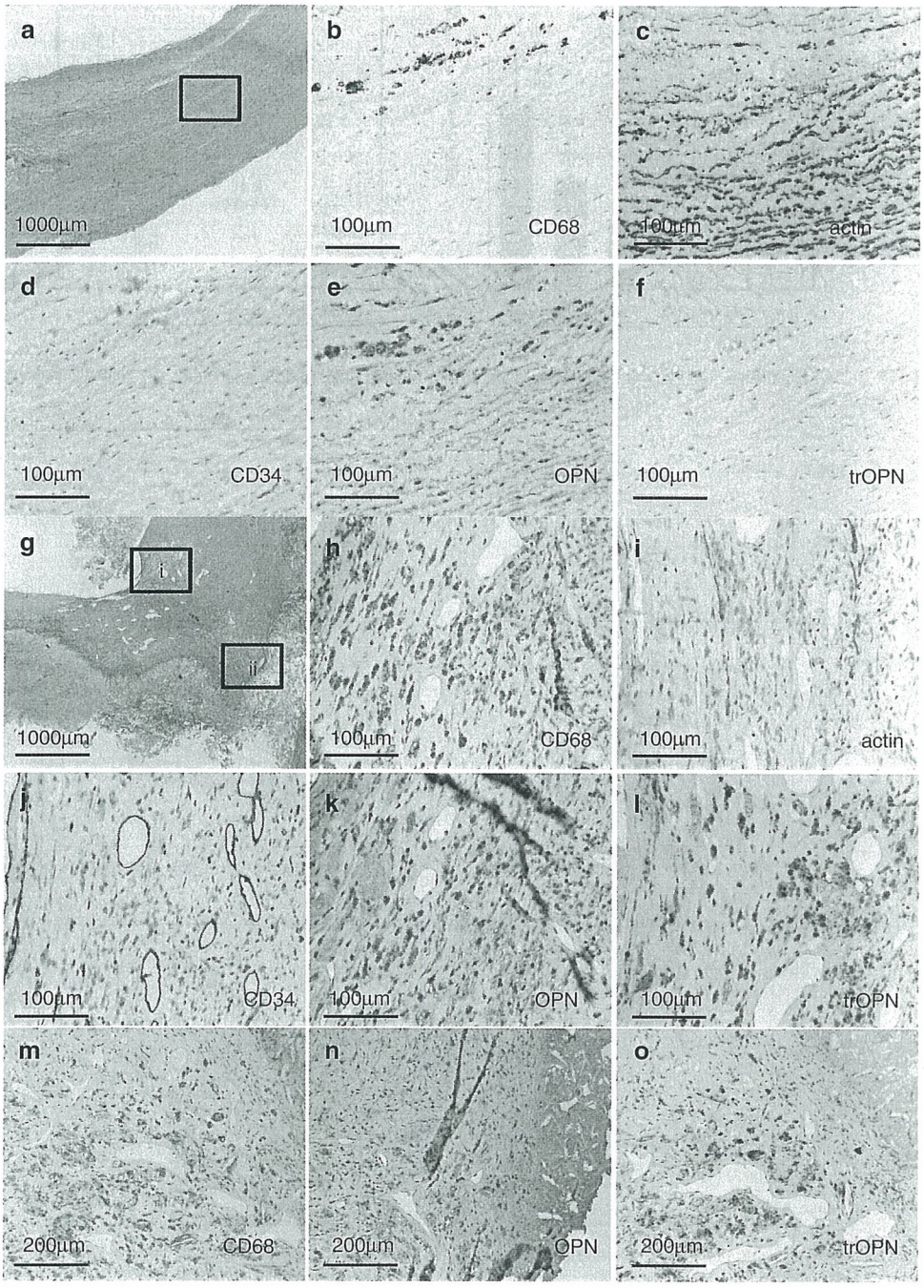

Figure 1 Representative immunohistochemistry of CEA specimens. (**a, g**); HE stain, (**b, h, m**); CD68, (**c, i**); αSMA, (**d, j**); CD34, (**e, k, n**); OPN, (**f, l, o**); trOPN. (**a**); Pathological intimal thickening. (**g**); Plaque with intraplaque hemorrhage; boxes i and ii are enlarged in (**h-l**) and (**m-o**), respectively. *OPN and trOPN within the extracellular matrix.

RESULTS

The presence of trOPN in atherosclerotic plaques was examined using Haematoxylin and Eosin (HE) stain and immunohistochemistry (IHC). OPN (Figures 1e, k and n) co-localized with CD68-positive cells, alpha smooth muscle actin (αSMA)-positive cells, lipid core and calcified nodules in a plaque that contained intraplaque hemorrhage, intraplaque vessels and a 'thin fibrous cap', as determined previously.[15] TrOPN was detected in intraplaque hemorrhages and partially co-localized with perivascular CD68-positive cells (Figures 1l and o). αSMA-positive cells in lesions with diffuse intimal thickening were OPN-positive, but trOPN was not detected (Figure 1f). OPN and trOPN was also detected in the debris on protection filters (Supplementary Figure 3).

EHT patients were compared with CAS patients to clarify the profile of OPN and trOPN levels (Supplementary Table 1). The CAS patients were older and more commonly male, with a higher prevalence of current smokers, lower body mass index, lower diastolic blood pressure, lower HDL-cholesterol, higher creatinine, higher HbA1c and higher triglycerides than EHT patients. Plasma OPN levels were divided into tertiles (low, middle and high). TrOPN levels

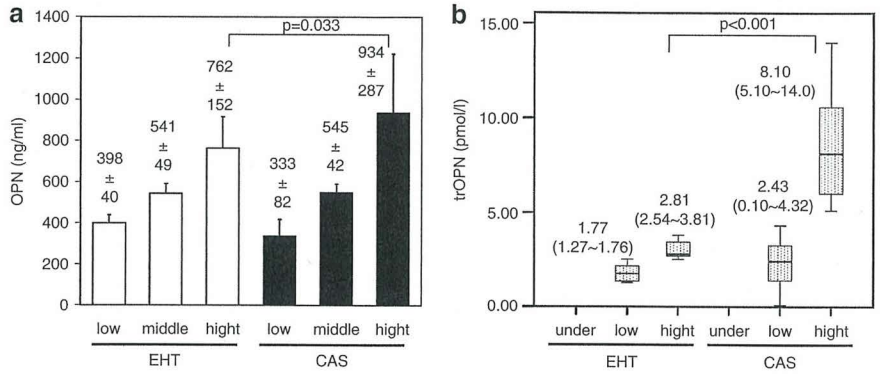

Figure 2 Comparison of EHT and CAS patients. (**a**) Plasma OPN levels divided into tertiles (low, middle and high). (**b**) TrOPN levels divided into two groups, under the detection limit (under) and others.

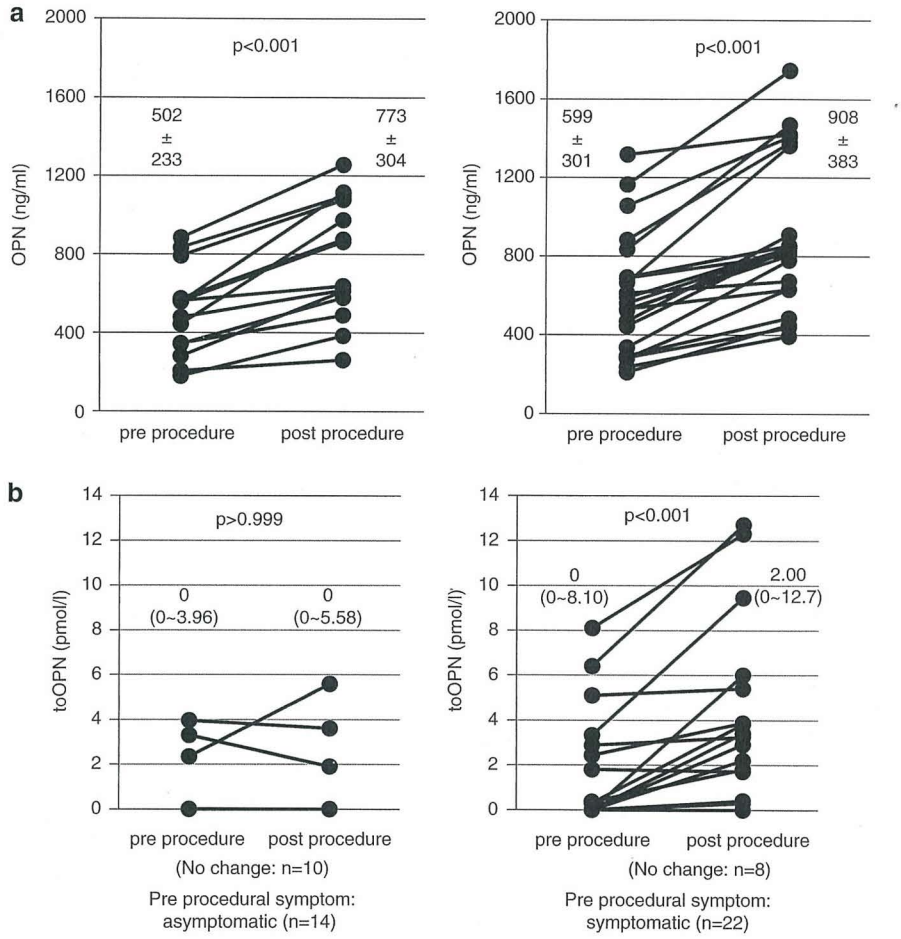

Figure 3 (**a**) Change in plasma OPN levels pre- and post-procedure. (**b**) Change in trOPN levels pre- and post-procedure. A, the mean ± s.d., paired *t* test, B, median (range), Wilcoxon test.

were not normally distributed, and the majority of trOPN levels were lower than the detection limit (n=62, 54.8%). The data were divided into three groups: beneath the detection limit, low trOPN and high trOPN levels. A small but significant difference between CAS and EHT patients was observed in the high OPN group (934 ± 287 ng ml^{-1} *vs.* 762 ± 151 ng ml^{-1}, respectively, P=0.032). Importantly, trOPN levels in the high CAS group were significantly higher than trOPN levels in the high EHT group (median 8.1 pmol l^{-1}, range 5.1–14.0, median 2.8 pmol l^{-1}, range 2.54–3.81, respectively, $P<0.001$) (Figure 2). The relationship between OPN, trOPN and other clinical parameters in CAS patients was analyzed (Supplementary Tables 2 and 3). Diabetes patients and bilateral carotid stenosis patients demonstrated higher OPN levels than the other patients (P=0.015, P=0.015, respectively). HDL-C was negatively correlated with trOPN, but creatinine and OPN were positively correlated with trOPN (ρ=−0.235, P=0.037, ρ=0.260, P=0.016, ρ=0.359, P=0.001, respectively).

Preoperative OPN and trOPN levels were compared in symptomatic (n=14) and symptomatic (n=22) CAS patients (Table 1). The

Table 2 Acute phase DWI after carotid artery stenting

	DWI negative (n=15)	*DWI positive (n=21)*	P-*value*
Pre-procedural symptom, yes (%)†	5 (33.3)	17 (80.9)	0.005
Diabetes mellitus (%)†	9 (60.0)	7 (33.3)	0.106
Ischemic heart disease (%)†	7 (46.7)	5(23.8)	0.110
ARB use (%)†	7 (46.7)	8 (38.0)	0.363
Statin use (%)†	6 (42.9)	8 (38.1)	0.526
Debris; high grade (%)	3 (20.0)	18 (85.7)	<0.0001
OPN pre (ng ml^{-1})	497 ± 210	619 ± 305	0.217
OPN post (ng ml^{-1})	719 ± 268	954 ± 385	0.070
Δ OPN (ng ml^{-1})	237 ± 123	334 ± 186	0.112
trOPN pre (pmol l^{-1})††	0 (0–3.96)	0 (0–8.10)	0.051
trOPN post (pmol l^{-1})††	0 (0–3.60)	2.2 (0–12.7)	0.003

Abbreviations: ARB; angiotensin II receptor blocker, DWI, diffusion-weighted cerebral MRIs; Δ OPN, post-OPN minus pre-OPN; OPN, osteopontin; trOPN, thrombin-cleaved form of osteopontin.
Statistical analysis was performed by Student's *t* test: †χ^2-test, ††Mann–Whitney's *U* test.

ratios of angiotensin I receptor blockers (ARB) use and BMI were lower in the symptomatic group. The mean duration of pre- and post-blood sampling was 2.7 ± 1.2 h. No differences in baseline OPN or trOPN levels between the two groups were observed (Figures 3a and b). Plasma OPN levels were elevated significantly in all patients after carotid artery stenting (asymptomatic, 502 ± 233 ng ml^{-1} to 773 ± 304 ng ml^{-1}, $P<0.001$; symptomatic, 599 ± 301 ng ml^{-1} to 908 ± 383 ng ml^{-1}, $P<0.001$, Figure 3a), but no difference in OPN increase was observed between the two groups (asymptomatic, 271 ± 149 ng ml^{-1}; symptomatic 181 ± 38 ng ml^{-1}, $P=0.519$). In contrast, plasma trOPN increased significantly in the symptomatic group post-procedure (median 0 to 2.0 pmol l^{-1}, $P<0.001$, Figure 3b), but no significant increase in the asymptomatic group was observed.

Post-operative DWI was analyzed to detect new ipsilateral cerebral ischemic lesions. A greater number of DWI-positive patients ($n=21$) were pre-operative symptomatic patients who demonstrated a higher grade of debris in the filter device and higher post-operative trOPN concentrations than DWI-negative patients ($P=0.005$, $P<0.001$, and $P=0.003$, respectively) (Table 2).

DISCUSSION

This study revealed the presence of trOPN in atherosclerotic plaques and demonstrated that plasma trOPN levels increased significantly after CAS in symptomatic stroke/TIA patients. Furthermore, patients with new ischemic cerebral lesions, as estimated by DWI, showed higher post-operative trOPN levels than patients with no new lesions. These findings support the presence of trOPN in highly thrombotic plaques and the use of trOPN as a plaque-derived biomarker for thromboembolic status.

TrOPN was identified in atherosclerotic plaques, especially hemorrhagic areas, using immunohistochemistry and colocalized with CD68-positive cells in intraplaque neovascularity. This report is the first to clarify the localization of trOPN in human atherosclerotic plaques. In our study, OPN was observed in macrophages, αSMA-positive cells, endothelial cells, lipid core and calcified nodules, as reported previously.[3] However, the distribution of trOPN was distinct. TrOPN was not observed in macrophage-rich areas in the absence of intraplaque vessels, which suggests a close relationship between trOPN and intraplaque hemorrhage and vessels.

TrOPN levels in CAS patients were significantly higher in the high trOPN group than trOPN levels in EHT patients (Figure 2). Because atherosclerosis is a systemic disease, extracranial carotid atherosclerosis frequently correlates with disease in other arteries, notably the aorta, coronary arteries and peripheral arteries.[16,17] This difference in OPN levels may reflect these situations in the two groups. Counter to our expectations, plasma OPN levels were only marginally higher in the CAS group compared with the EHT group. One reason for this difference could be the use of medication, because both atorvastatin and ARB decrease plasma OPN levels.[18,19] The difference in the relationships between OPN or trOPN and other clinical backgrounds (Supplementary Tables 2 and 3) suggests that different mechanisms regulate plasma OPN and trOPN levels.

The significant post-procedural increase in plasma trOPN levels in symptomatic patients was probably due to the protrusion of plaque contents that contained high levels of trOPN. Cholesterol crystals were evident in the filter protection devices, which supports the hypothesis that the disruption of plaques during the procedure releases plaque content into the circulation. The debris content in symptomatic patients was potentially different from asymptomatic patients. Alternatively, aortic plaques may be the source of trOPN-containing plaque contents. Rosenkranz *et al.* reported that embolus signals, which were estimated using transcranial Doppler, were detected during the CAS procedure before the involvement of the target lesion.[17] This result suggests that the aortic arch and the proximal supra-aortic arteries may detach debris fragments or platelet aggregates from the endothelium or atherosclerotic plaques. Symptomatic stroke patients have complex plaques in the proximal descending aorta, and these plaques constitute an underestimated embolic cause of stroke.[20] In support of these reports, symptomatic patients in our study demonstrated a higher thrombotic/embolic status than asymptomatic patients. Although blood pressure did not differ between the two groups, ARB use was lower in symptomatic patients than asymptomatic patients. Imanishi *et al.* reported that the combined treatment with an HMG-CoA reductase inhibitor and an ARB reduces atherosclerotic plaque formation and increases nitric oxide production.[21] The clinical use of ARB decreases future cardiovascular events.[22] These additive protective effects of ARB on endothelial function might occur in asymptomatic patients and stabilize the plaque to improve post-procedural trOPN levels.

In contrast, the increase in plasma OPN levels was similar in symptomatic and asymptomatic patients, and the increase in OPN was not correlated with trOPN levels. Different mechanisms may contribute to changes in OPN and trOPN levels. Heparin was injected before the CAS procedure in all patients. Heparin is an anticoagulant that inhibits the activation of thrombin. Heparin inhibits thrombin cleavage of OPN *in vitro*.[23] In our study, heparin might have suppressed plasma OPN cleavage by thrombin, which may explain why the increase in OPN levels did not correlate with post-procedural trOPN levels. Endothelial cells also produce OPN,[3] and the stress of the catheter and contrast medium could have induced OPN secretion from endothelial cells. Other inflammatory cytokines, such as interleukin-6, matrix metalloproteinases 3 and 9, high-sensitivity CRP and tumor necrosis factor, were also measured as reported previously.[24] No immediate post-procedural alterations in these cytokines were observed, but an increase in cytokines was observed the following day ($n=10$, data not shown). DWI-MRI monitored post-procedural outcomes. The majority of post-stent DWI-positive patients had high-grade debris in the protection filter ($n=18$, 85.7%), and their post-stent trOPN levels were higher than DWI-negative patients. These results support our hypothesis of a close relationship between trOPN levels and thrombotic status. Pretreatment trOPN levels were not significantly different between DWI-negative and DWI-positive

groups. A lack of statistical power may explain this result because trOPN levels were undetectable in 24 patients. This result may also be due to the selection of CAS or CEA. Surgeons selected CEA for plaques with a large thrombus burden, ulceration and frequent ischemic attacks, which were estimated by ultrasound or MRI.[11] One emergent coronary artery bypass graft was performed 6 months after CAS, and one patient had a new bilateral DWI lesion after CAS in the group of patients with high trOPN levels ($>2.8\,\mathrm{pmol\,l^{-1}}$, $n=5$, Supplementary Table 3) bfore surgery. Although the current study was relatively small, the results suggest that high trOPN levels before CAS reflect a systemically unstable situation.

This study suffers from some limitations. First, EHT patients with no symptomatic disease were compared with CAS patients, but a clarification of trOPN levels that constitute abnormal levels could not be established. Second, although thrombus was estimated, other coagulation factors were not measured. Third, imaging assessments before the treatment were not uniform, and precise data of the severity of carotid stenosis were not obtained. Therefore, the relationship between plaque volumes and plasma OPN or trOPN levels was difficult to assess. Fourth, the number of participants was small, which weakened the statistical power. Further studies that include a larger population are required.

The function of trOPN in human atherosclerotic plaques is not clear. However, trOPN colocalized with CD68-positive macrophages in our study. Yamamoto *et al.* reported that splenic monocytes from arthritic mice exhibited a significant capacity for cellular migration toward trOPN but not toward full-length OPN.[25] A specific murine monoclonal antibody to a cryptic epitope of human OPN ameliorated established collagen-induced arthritis in cynomolgus monkeys,[25] which suggests that trOPN is an important accelerator of inflammation in humans. Further experimental studies and prospective clinical studies are required to investigate these possibilities.

CONCLUSION

There was a significant increase in TrOPN levels after carotid artery stenting in patients with pre-operative symptoms. TrOPN may be a novel index of cardiovascular diseases as a biomarker for both atherosclerotic and thrombotic status.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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①Cerebrospinal Fluid Enhancement on Fluid Attenuated Inversion Recovery Images After Carotid Artery Stenting With Neuroprotective Balloon Occlusions: Hemodynamic Instability and Blood-Brain Barrier Disruption.,

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Cerebrospinal Fluid Enhancement on Fluid Attenuated Inversion Recovery Images After Carotid Artery Stenting with Neuroprotective Balloon Occlusions: Hemodynamic Instability and Blood–Brain Barrier Disruption

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Abstract
Purpose A rare complication of carotid artery stenting (CAS), prolonged reversible neurological symptoms with delayed cerebrospinal fluid (CSF) space enhancement on fluid attenuated inversion recovery (FLAIR) images, is associated with blood–brain barrier (BBB) disruption. We prospectively identified patients who showed CSF space enhancement on FLAIR images.
Methods Nineteen patients—5 acute-phase and 14 scheduled—underwent 21 CAS procedures. Balloon catheters were navigated across stenoses, angioplasty was performed using a neuroprotective balloon, and stents were placed with after dilation under distal balloon protection. CSF space hyperintensity or obscuration on FLAIR after versus before CAS indicated CSF space enhancement. Correlations with clinical factors were examined.
Results CSF space was enhanced on FLAIR in 12 (57.1%) cases. Postprocedural CSF space enhancement was significantly related to age, stenosis rate, acute-stage procedure, and total occlusion time. All acute-stage CAS patients showed delayed enhancement. Only age was associated with delayed CSF space enhancement in scheduled CAS patients.
Conclusions Ischemic intolerance for severe carotid artery stenosis and temporary neuroprotective balloon occlusion, causing reperfusion injury, seem to be the main factors that underlie BBB disruption with delayed CSF space enhancement shortly after CAS, rather than sudden poststenting hemodynamic change. Our results suggest that factors related to hemodynamic instability or ischemic intolerance seem to be associated with post-CAS BBB vulnerability. Patients at risk for hemodynamic instability or with ischemic intolerance, which decrease BBB integrity, require careful management to prevent intracranial hemorrhagic and other post-CAS complications.

Keywords Cerebrospinal fluid · Fluid attenuated inversion recovery image · Carotid artery stenting · Blood–brain barrier · Cervical internal carotid artery stenosis

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Introduction

Carotid artery stenting (CAS) is emerging as a potential alternative to carotid endarterectomy (CEA). Most complications after CAS are ischemic in nature and are attributable to embolization or inadequate cerebral protection in patients with poor collateral blood supply. In addition, postoperative hyperperfusion syndrome and intracranial hemorrhage have been extensively described as relatively uncommon but with devastating complications following CAS [1–5]. Recently, we reported the rare post-CAS complication of prolonged reversible neurological symptoms associated with delayed enhancement of the cerebrospinal fluid (CSF) space on fluid attenuated inversion

recovery (FLAIR) images [6]. Although this phenomenon, developed after vascular reconstruction therapy, is associated with blood–brain barrier (BBB) disruption resulting from reperfusion injury or sudden hemodynamic change, its interpretation remains controversial due to the very small number of studies focusing on post-CAS delayed CSF enhancement. To determine the incidence, as well as clinical and background factors predicting delayed CSF enhancement on FLAIR images following CAS, we performed a prospective study at our institution to identify patients who developed CSF space enhancement on FLAIR images.

Patients and Methods

We enrolled 19 consecutive patients (21 lesions; 6 women, 13 men; 11 symptomatic, 10 asymptomatic; mean age 74 ± 8 years) who underwent CAS between September 2006 and June 2007. CAS was performed in accordance with accepted surgical indications in asymptomatic patients with ≥70% stenosis of the extracranial carotid artery and symptomatic individuals with ≥60% stenosis of this artery. The diameter of the stenotic lesions was determined according to the North American Symptomatic Carotid Endarterectomy Trial (NASCET) criteria [7]. Five (6 lesions) of the 19 patients, with unstable or deteriorating ischemic symptoms attributable to a stenosis of the ipsilateral carotid artery without a large hyperintense lesion on diffusion weighted imaging (DWI) and with a large area of hypoperfusion on perfusion weighted image (PWI), underwent the CAS procedure within 2 weeks after the onset. We defined these patients as having received an "acute stage procedure."

The study was approved by the Local Research Ethics Committee of Mazda Hospital, and all patients provided written, informed consent after receiving a full explanation of the goals, methods, and risks of the study.

Procedure

Carotid Artery Stenting

For patients with unstable or deteriorating ischemic symptoms, acute stage CAS was performed under general anesthesia because these patients frequently move, occasionally even resisting medical intervention, during surgery if only local anesthesia is used. All patients who received general anesthesia, were administered Propofol, rocuronium, and opioids intravenously. Scheduled CAS procedures were performed under local anesthesia with sedation using a low dose of Propofol. Patients were pretreated with aspirin 100 mg per day and ticlopidine 200 mg per day for at least 1 week before the CAS procedure. In cases that required acute stage intervention, sodium ozagrel 160 mg per day, was given by drip infusion along with the aforementioned medications. The activated clotting time was maintained at more than twice the baseline value throughout all procedures by continuously infusing intravenous heparin. In 12 lesions with severe (≥90%) stenosis, a PATLIVE guiding catheter (Terumo Clinical Supply Co., Ltd., Gifu, Japan) and a PercuSurge device (Medtronic, Inc., Minneapolis, MN) were introduced into the common carotid artery and the external carotid artery, respectively. A percutaneous transluminal angioplasty (PTA) balloon catheter was navigated across the stenosis with another PercuSarge device, and balloon angioplasty was performed under proximal balloon protection using the PATLIVE guiding catheter and PercuSurge device at the external carotid artery. The stent was then placed and postdilation was performed under distal balloon protection using the PercuSarge device at the internal carotid artery. In nine lesions with ≤90% stenosis, all three steps were performed under distal protection using the PercuSarge device at the internal carotid artery. The mean total occlusion time using the neuroprotective balloon was 11.9 ± 3.4 min. The stent diameter was 7.0 mm for two lesions, 8.0 mm for four lesions, 9.0 mm for eight lesions, and 10 mm for seven lesions.

Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) examinations were performed using a 1.5T system (GE Medical Systems, Milwaukee, WI). The full MRI protocol consisted of a variety of sequences used to acquire data before and after stenting. Herein, we discuss only a portion of the imaging results in detail. A fast FLAIR sequence (TE = 114 ms; TR = 8000 ms; inversion time = 2000 ms; echo train length = 18, acquisition matrix 128 × 224 over a 24-cm FOV; average = 1) was used to acquire 20 6-mm in thickness contiguous axial images. These images were acquired before and after intravenous administration of 15 ml of the contrast agent GD-DTPA (OMNISCAN, Daiichi-Sankyo Co. Ltd.).

The mean time between administration of the Gd-DTPA and insertion of the stent was 57 (range, 10–165) min. The mean time between stent insertion and the poststenting MRI was 18.1 (range, 10–25) h.

Three observers (RO, OH, and TN) judged the MRI independently. Hyperintensity or obscuration of the CSF space on postoperative compared with preoperative FLAIR images was identified as enhancement of the CSF space.

Data Collection and Statistical Analysis

All cases were categorized based on the presence or absence of CSF space enhancement on postprocedural FLAIR images. The following clinicopathological factors

were studied: age, sex, concomitant disease states (e.g., hypertension, diabetes mellitus, and hyperlipidemia), degree of internal carotid artery stenosis, scheduled versus acute stage procedure, duration from administration of Gd-DTPA to insertion of the stent, duration of neuroprotective balloon occlusion, duration from stent insertion to the poststenting MRI, presence of an associated ischemic lesion on postoperative DWI, and occurrence or exacerbation of neurological symptoms. By definition, acute stage procedures were performed within 2 weeks after the last ischemic event. The relationship between each of these factors and the occurrence of postprocedural CSF space enhancement was evaluated by univariate analysis using the Mann–Whitney *U* test or the Fisher exact test. Differences were deemed statistically significant if the probability value was <0.05.

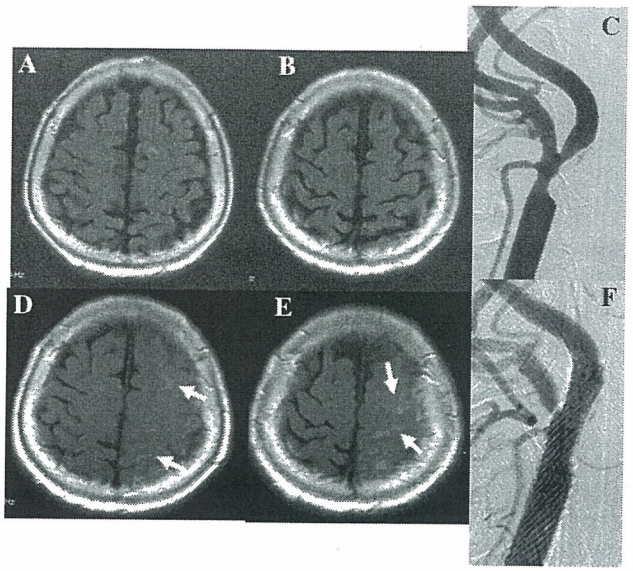

Fig. 1 Sample images of the same anatomic slice from a patient who underwent a scheduled stent procedure that showed CSF space enhancement within portions of the left frontal and parietal lobes, ipsilateral to the stented artery **A**, **B** FLAIR image before stenting; **C** conventional angiogram of the carotid bifurcation after stenting; **D**, **E** FLAIR image after stenting showing hyperintensity of the CSF space compared with the preoperative FLAIR image (*arrow*); **F** conventional angiogram of the carotid bifurcation after stenting

Results

All 21 stent insertions were successfully completed with ≤30% residual stenosis. Two patients experienced postprocedural ischemic symptoms, but these had resolved by the time of hospital discharge and no patients had permanent neurological deficits associated with the CAS procedure.

In 12 (57.1%) of the 21 procedures, areas of CSF space enhancement on FLAIR images after stent placement were demonstrated. All enhanced areas were ipsilateral to the stented carotid artery (Fig. 1). All CSF space enhancements were distributed within the MCA territory at the core of the watershed area between the MCA and the PCA. Only three of these lesions were recognized in the watershed area between the MCA and the ACA. No enhancements were detected in the basal cistern. In all 21 cases, computed tomography (CT) immediately after stent insertion revealed no obvious hemorrhagic change or any obvious leptomeningeal enhancement. Furthermore, neither intracranial hemorrhage nor cerebral hyperperfusion syndrome occurred after the CAS procedure.

Table 1 shows the relationships between clinicopathological factors and the development of postprocedural CSF space enhancement. Univariate analysis revealed the following variables to be significantly related to postprocedural CSF space enhancement: age ($P \leq 0.05$), rate of stenosis ($P \leq 0.01$), acute stage procedure ($P \leq 0.01$), and total occlusion time ($P \leq 0.05$). Most notably, all patients who underwent an acute stage procedure for carotid artery stenosis, who had unstable or deteriorating ischemic symptoms, showed delayed enhancement on postprocedural FLAIR images (Fig. 2).

Table 2 shows the results of a univariate analysis of factors related to the appearance of delayed CSF space enhancement in patients undergoing a scheduled CAS procedure. The only variable significantly associated with the appearance of delayed CSF space enhancement was age.

Discussion

The integrity of the BBB can be evaluated noninvasively using MRI after intravenous administration of Gd-DTPA, which does not cross the intact BBB. The findings of T1-weighted imaging with contrast medium in animal models of stroke indicate pathology and correlate with the severity of BBB disruption. These findings can thus predict subsequent hemorrhagic transformation. Extravasation of contrast medium into the CSF results in shortening of the T1 relaxation time of CSF, poor fluid suppression on FLAIR imaging, and hyperintensity of the CSF space. The contrast medium is believed to cross the BBB, enter the perivascular space, and then distribute into the CSF via a perivascular pathway, in a fashion analogous to the movement of horseradish peroxidase seen in earlier studies. The FLAIR technique, being a "null method," is 10 times more sensitive than T1-weighted imaging to the concentration of contrast medium [8]. The short plasma distribution and elimination half-lives of Gd-DTPA, 0.2 ± 0.13 and 1.6 ± 0.13 h, respectively, indicate that the BBB disruption occured before or soon after intravenous

Table 1 Univariate analysis of the relationship between clinicopathological factors and the development of postprocedural enhancement of the CSF space in all patients

Factor of patient	With CSF space enhancement ($n = 11$)	Without CSF space enhancement ($n = 10$)	P value
Age ± SD (yr)	77.7 ± 8.1	70.3 ± 5.4	0.0481[a]
Age range (yr)	67–93	62–82	
Male gender	8 (72.7)	7 (70)	1
Rate of stenosis ± SD	86.2 ± 12.6	69.3 ± 13.5	0.0089[a]
Hypertension	10 (90.9)	8 (80)	0.5865
Diabetes mellitus	4 (36.4)	2 (20)	0.6351
Hypercholesterolemia	5 (45.5)	3 (30)	0.6594
Postprocedural ischemic symptom	2 (18.2)	0 (0)	0.4792
Contralateral lesion (≥80%)	2 (18.2)	0 (0)	0.4792
Acute stage procedure	6 (54.5)	0 (0)	0.0124[b]
Total occlusion time by protection device (min)	13.7 ± 3	9.9 ± 3	0.0112[a]
Time between the administration of Gd-DTPA and stent insertion ± SD (min)	77.3 ± 60.3	35.7 ± 28.5	0.1171
Time between the stent insertion and postoperative MRI ± SD (h)	19.7 ± 3.2	20.4 ± 4	0.5654

Data are numbers with percentages in parentheses unless otherwise indicated

CSF cerebrospinal fluid, *Gd-DTPA* gadolinium diethylenetriamine pentaacetic acid, *MRI* magnetic resonance imaging, *SD* standard deviation

[a] Calculated using nonparametric Mann–Whitney *U* test

[b] Calculated using Fisher's exact test

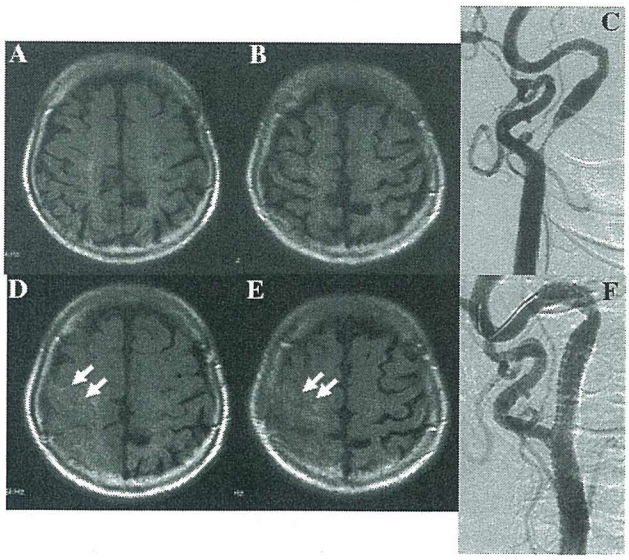

Fig. 2 Sample images of the same anatomic slice from a patient who underwent a scheduled stent procedure that showed CSF space enhancement within portions of the left frontal and parietal lobes, ipsilateral to the stented artery **A**, **B** FLAIR image before stenting; **C** conventional angiogram of the carotid bifurcation before stenting; **D**, **E** FLAIR image after stenting showing obscuration of the CSF space compared with the preoperative FLAIR image (*arrow*); **F** conventional angiogram of the carotid bifurcation after stenting

administration of this contrast agent. On the other hand, delayed gadolinium enhancement of the CSF space on FLAIR images is a marker of early BBB disruption and can be seen as late as 5 days after Gd-DTPA administration [9].

An adverse reaction to nonionic iodinated contrast medium also may result in BBB disruption after CAS. Nonionic contrast medium is widely used for intravascular neurointerventional procedures. Several clinical complications were suspected to be related to disruption of the BBB by nonionic contrast medium [10–13]. These cases reportedly showed abnormal enhancement in the cerebral cortex on CT after angiography. Postprocedural CT demonstrated no abnormalities in any of the cases in our study, suggesting that the nonionic contrast medium did not disrupt the BBB. In addition, it seems unlikely that systemic intravenously administered drugs, such as Propofol, rocuronium, opioids, etc. were the cause, because all areas of CSF space enhancement were visualized only on the side ipsilateral to the stented carotid artery. Furthermore, Fischer et al. although based on an in vitro study, reported that anesthesia involving Propofol does not alter the permeability of the BBB [14].

Wilkinson et al. reported that MRI demonstrated unilateral leptomeningeal enhancement in several cases at approximately 2 h after CAS, and this appeared to be directly related to altered BBB integrity following sudden hemodynamic change created by the stenting [15]. Furthermore, in our previous report, we raised the possibility of BBB disruption after CAS, including neuroprotective balloon occlusions being associated with inflammatory injury secondary to a lack of tolerance for sudden hemodynamic change or the neuroprotective balloon occlusions

Table 2 Univariate analysis of the relationship between clinicopathological factors and the development of postprocedural enhancement of the CSF space in patients who underwent scheduled procedures.

Factor of patient	With CSF space enhancement ($n = 5$)	Without CSF space enhancement ($n = 10$)	P value
Age ± SD (yr)	79.4 ± 7.4	70.3 ± 5.4	0.0492[a]
Age range (yr)	69–87	62–82	
Male gender	8 (72.7)	7 (70)	1
Rate of stenosis	74.8 ± 9.7	69.3 ± 13.5	0.354
Hypertension	5 (80)	8 (80)	1
Diabetes mellitus	3 (60)	2 (20)	0.2507
Hypercholesterolemia	2 (40)	3 (30)	1
Postprocedural ischemic symptom, n(%)	1 (20)	0 (0)	0.3333
Contralateral lesion (≥80%)	0 (0)	0 (0)	1
Total occlusion time by protection device ± SD (min)	12.8 ± 3	9.9 ± 3	0.0662
Time between the administration of Gd-DTPA and stent insertion ± SD (min)	47 ± 28	35.7 ± 28.5	0.6139
Time between the stent insertion and postoperative MRI ± SD (h)	21.6 ± 2.7	20.4 ± 4	0.6214

Data are numbers with percentages in parentheses unless otherwise indicated

CSF cerebrospinal fluid, *Gd-DTPA* gadolinium diethylenetriamine pentaacetic acid, *MRI* magnetic resonance imaging, *SD* standard deviation

[a] Calculated using nonparametric Mann–Whitney *U* test

themselves [6]. Studies in animal reperfusion injury models using protein tracers to assay microvascular integrity have shown that BBB disruption can occur in two distinct phases. The initial transient disruption is considered to be "hemodynamic" in nature. It would presumably be caused by reperfusion, loss of autoregulation and reactive hyperemia. More recent studies have implicated matrix metalloproteinases that attack the basal lamina and later result in damage to the ultrastructure of the microvasculature [8, 16]. In addition, numerous indirect mechanisms exacerbating microvascular structure damage include endothelial activation, excess production of oxygen-free radicals, inflammatory responses, leukocyte recruitment, increased cytokine production, and edema formation. All of these mechanisms involve concomitant changes in microvascular structure. Molecular adhesive events and cytokine production occur early in ischemia and reperfusion and underlie the transition from ischemic to inflammatory injury. The subsequent recruitment of leukocytes to the ischemic zone may lead to reocclusion of microvessels [9, 16].

In the present study, we demonstrated factors related to the incidence of ischemic intolerance, such as age, rate of stenosis, acute stage procedure, and total occlusion time, to be significantly associated with the development of delayed CSF space enhancement in all patients. In addition, such phenomena mainly occurred in the watershed area between the MCA and the PCA ipsilateral to the stented carotid artery. These findings suggest that changes in BBB integrity occur in areas that tend to be under low arterial pressure and have low flow rates as a result of severe carotid artery stenosis or temporary neuroprotective balloon occlusion. Furthermore, we demonstrated age to be the only factor significantly associated with the development of delayed CSF space enhancement in scheduled CAS patients. Therefore, it is reasonable to speculate that reperfusion injury resulting from ischemic intolerance for severe carotid artery stenosis or temporary neuroprotective balloon occlusion may be the main factor responsible for BBB disruption with the delayed CSF space enhancement developing approximately half a day after the CAS procedure, rather than sudden hemodynamic change following stenting.

It is noteworthy that patient age was significantly associated with the development of delayed CSF space enhancement in both the entire group of CAS patients and those undergoing scheduled CAS. In previous studies, BBB function and CSF flow were shown to decrease with aging. These decreases in BBB function and CSF flow may account for the delayed CSF space enhancement often seen in elderly patients [17–19].

Warach and Latour noted that early BBB disruption represented by Gd-DTPA extravasation after recanalization therapy for an occluded intracerebral artery was associated with hemorrhagic transformation and with poor outcomes [9]. None of the patients in our study showed obvious intracerebral hemorrhage or permanent neurological deficits. Based on our results, however, factors related to the incidence of hemodynamic instability or ischemic intolerance, such as advanced age, higher rate of stenosis, acute stage procedure, and longer total occlusion time, may be associated with vulnerability of the BBB following CAS. In other words, performing the CAS procedure in patients at risk for hemodynamic instability or with ischemic

intolerance may tend to decrease BBB integrity, as well as result in intracerebral hemorrhage and other adverse outcomes. Therefore, we suggest that strict control of blood pressure and more aggressive sedation, including general anesthesia, should be considered in such patients to prevent intracranial hemorrhagic complications or other adverse outcomes following CAS. Furthermore, if this hypothesis holds true, pharmacologically disrupting the BBB may facilitate the prevention of hemorrhagic complications after CAS.

Hyperperfusion syndrome is a rare complication of cervical recanalization procedures, including CAS and CEA, and usually presents as transient focal deficits associated with migraine-like headache, seizures, and intracerebral hemorrhage. With CEA, hyperperfusion syndrome is a known clinical complication that generally manifests 5–8 days after the procedure [15]. In contrast, recent studies on hyperperfusion syndrome and intracranial hemorrhage after CAS demonstrated the onsets of hyperperfusion syndrome and hemorrhagic transformation to peak within 12 h after the procedure [20]. The difference between the two procedures in terms of the timing of hyperperfusion syndrome and intracranial hemorrhage onset is controversial. The present study demonstrated that enhancement occurred in approximately 60% of patients after CAS. Although, to the best of our knowledge, no studies have focused on delayed CSF space enhancement after CEA, the difference between the two procedures regarding postprocedural BBB vulnerability may explain the difference between the two procedures in the timing of the onsets of hyperperfusion syndrome and intracranial hemorrhage. Future investigations on BBB disruption resulting from sudden hemodynamic change and reperfusion injury after vascular reconstruction therapy are necessary.

Conflict of interest The authors declare that they have no conflict of interest.

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