family is interviewed after the TIA attack. A total of 67 patients with left hemispheric involvement were finally enrolled. These patients consisted of 47 men and 20 women aged 65.7±10.3 years. A diagnosis of carotid TIA was made by neurologists based on the National Institute of Neurological Disorders and Stroke classification III (4). Patients with amaurosis fugax were also excluded from this study.

Computed tomography (CT) was performed on all patients within two weeks of onset of the TIA in order to exclude nonischemic brain lesions such as brain hemorrhage, chronic subdural hematoma, and brain tumors.

Information on symptoms during the TIAs was obtained from the patients or their families. If a patient had a speech disturbance, a careful history was taken so as to distinguish aphasia from dysarthria. Two of the authors (K. W. and K. Y.), who were blinded to the patients' clinical and laboratory data, independently assessed the TIA symptoms documented in the admission records and classified the patients into three subgroups according to TIA symptoms: 1) the aphasia group, patients having an aphasia with or without paresis; 2) the monoparesis group, those presenting with an isolated motor or sensory disturbance restricted to either the face, arm or leg; and 3) the hemiparesis group, those presenting with hemiparesis or hemisensory deficits without aphasia. When the two investigators disagreed on a patient's classification, they discussed the case and reached an agreement on the final classification.

The following baseline and clinical characteristics were evaluated: 1) age and gender: 2) duration of TIA (<1 hour, and 1–24 hours): 3) number of TIAs: 4) use of antiplatelet agents or anticoagulants: 5) past history of brain infarction, TIA, myocardial infarction, or definite angina pectoris: 6) risk factors for stroke, including hypertension, diabetes mellitus, hyperlipidemia, and current smoking: 7) significant arterial pathologies in the left carotid system: and 8) potential cardiac sources of emboli.

The criteria for stroke risk factors were as follows: 1) use of antihypertensive agents, systolic blood pressure (SBP) >160 mmHg or diastolic blood pressure (DBP) >95 mmHg: 2) use of oral hypoglycemic agents, insulin, or glycosylated hemoglobin (HbA1C) >6.4%: 3) use of antihyperlipidemic agents, or serum cholesterol level >220 mg/dl: and 4) current smoking defined as a history of smoking in the preceding three months.

To detect potential cardiac sources of emboli (emboligenic cardiac diseases), all patients underwent 12-lead electrocardiography (ECG), 24-hour ECG monitoring, and transthoracic echocardiography. AF included both paroxysmal and persistent AF and was identified during hospitalization. Emboligenic cardiac diseases included non-valvular AF: acute myocardial infarction, old myocardial infarction with intraventricular thrombus: mitral valve disease: prosthetic cardiac valve: implantation of a pacemaker: and dilated cardiomyopathy.

We performed color-flow duplex carotid ultrasonography, conventional angiography, and MR angiography (MRA) in order to evaluate significant arterial pathologies in the ipsilateral carotid system. Color-flow duplex carotid ultrasonography (Toshiba SSA 270A or 260A, Toshiba Inc, Tokyo) was performed to assess the extracranial carotid stenotic lesions in all patients. The carotid arteries were examined in longitudinal and transverse planes from anterior, lateral, and posterior approaches using B-mode and color flow imaging. This allowed direct measurement of the residual lumen diameter of the stenotic lesions.

We performed conventional cerebral angiography in 39 patients (58%), MRA in 30 (45%), and both assessments were done in 7 patients (10%). Therefore, in 62 patients (93%) the intra-cranial carotid artery, anterior cerebral artery and middle cerebral artery were assessed by either conventional cerebral angiography or MRA. The grade of stenosis of the internal carotid artery (ICA) was determined by the method used in the North American Symptomatic Carotid Endarterectomy Trial (5). Stenosis in the middle cerebral artery (MCA) was calculated in a similar manner by measuring the diameter of the stenotic lesion and that of an adjacent intact portion of the artery.

The lesions were considered significant if the horizontal portion of the MCA had a >50% stenosis, and the ICA was a >70% stenosis or if an ulceration was evident in the carotid bifurcation.

The risk factors, past history, arterial or cardiac diseases, number of TIAs, and TIA duration were compared between the two groups using the χ^2 test. Patient age was analyzed using the Mann-Whitney U test. Statistical analysis was performed using a commercially available software package (Stat-View, version 5, SAS Institute Inc. USA). Data were expressed as mean±SD. P values <.05 were considered statistically significant.

Results

Twelve patients had AF (the AF group) and 55 did not have AF (non-AF group). Table 1 shows the clinical characteristics of these two groups. The mean of age in the AF group was higher than in the non-AF group (p=0.014). No significant differences between the two groups were observed in: the duration or number of TIAs: use of medication: past history: or risk factors. Arterial diseases were seen more frequently in the non-AF group than in the AF group (53% vs 17%, p=0.028)

Among the 62 patients evaluated with color-flow duplex carotid ultrasonography and conventional angiography or MRA, significant arterial disease was detected in the carotid axis ipsilateral to the affected side in 30 (45%) patients. The following arterial lesions were observed: MCA occlusion in 4 patients, anterior cerebral artery occlusion in 1 patient, more than 50% stenosis of the horizontal portion of the MCA in 9 patients, ICA occlusion in 2 patients, more than 70% ICA stenosis in 10 patients, and less than 70% ICA stenosis but with ulceration in 4 patients. In 1 of the 4 patients who did not have conventional angiography or MRA

Table 1. Demographic Features of TIAs in the 2 Groups

	AF Group n=12	Non-AF Group n=55	p
Age, years	73.0±9.7	64.1±9.8	0.014
TIA duration, n			N.S
<1 hour	6 (50%)	32 (58%)	
1 hour–24 hours	6 (50%)	23 (42%)	
Number of TIAs, n			N.S
1	10 (83%)	35 (64%)	
2–3	2 (17%)	12 (22%)	
4–9	0	8 (15%)	
Medication at TIA, n (%)			
Anticoagulation	2 (17%)	1 (2%)	N.S
Antiplatelet	2 (17%)	13 (24%)	N.S
Past history, n (%)			
TIA	3 (25%)	10 (18%)	N.S
Brain infarction	4 (33%)	11 (20%)	N.S
Angina pectoris	0 (0)	4 (7%)	N.S
Myocardial infarction	1 (8%)	3 (5%)	N.S
Risk factor, n (%)			
Hypertension	5 (42%)	39 (71%)	N.S
Diabetes mellitus	2 (17%)	17 (31%)	N.S
Hyperlipidemia	2 (17%)	26 (47%)	N.S
Smoking	6 (50%)	28 (51%)	N.S
Arterial disease	2 (17%)	29 (53%)	0.028
Emboligenic cardiac diseases			
Others except for AF	0	4 (7%)	N.S

N.S: not significant.

Table 2. Clinical TIA Symptoms of the 2 Groups

	AF Group n=12	Non-AF Group n=55	p
Hemiparesis without aphasia	2 (17%)	30 (55%)	0.025
Monoparesis without aphasia	2 (17%)	14 (25%)	0.517
Aphasia	8 (67%)	11 (20%)	0.003

examinations, color-flow duplex carotid ultrasonography demonstrated a 75% stenosis in the internal carotid artery in one patient. Thus a total of 31 patients had significant arterial diseases (Table 1).

Emboligenic cardiac diseases were observed in 16 (24%) patients: 12 patients had non-valvular AF: 2 had a prosthetic mitral valve: 1 had a pacemaker: and 1 had an acute myocardial infarction. Other than AF, there were no significant differences observed in emboligenic cardiac diseases between the two groups (Table 1).

Overall, 19 patients (28%) had transient aphasia with either hemiparesis or monoparesis, 16 (24%) had monoparesis without aphasia, and 32 (48%) had hemiparesis without aphasia (Table 2). Aphasia was observed more frequently in the AF group than in the non-AF group (67% vs 20%, p=0.003). Hemiparesis without aphasia was seen less frequently in the AF group than in the non-AF group (17% vs 55%, p=0.025).

Discussion

The present study showed that TIA patients with AF had aphasia more frequently than TIA patients without AF. Mohr et al (6, 7) reported that some patients with cardiogenic embolism showed an abrupt onset of a major hemispheric symptom, such as aphasia, hemianopia, or unilateral spatial neglect, which was followed within hours to days by a disappearance of most of the clinical manifestations. This phenomenon of rapid recovery was termed "a spectacular shrinking deficit (SSD)". Minematsu et al (8) reported that the SSD was closely related to cardiogenic brain embolism. TIAs with a major hemispheric symptom partly overlap with SSD, and therefore may be related to cardiogenic brain embolism. A cardiogenic embolus may be larger than an embolus from arterial disease. Therefore, patients with AF might have a transient embolic occlusion in the larger vessels, which leads to major hemispheric symptoms such as aphasia.

In the present study, hemiparesis without aphasia was more frequently found in the non-AF group than in the AF group. The etiology of the TIAs in the AF group is likely be cardioembolic. In TIA patients without emboligenic cardiac diseases, Hankey and Warlow (9) have proposed that the TIAs could be classified into lacunar and cortical TIA syndromes according to the clinical symptoms during the attack. Cortical TIA syndromes are associated with atheromatous disease in the ipsilateral extracranial ICA. On the other hand, lacunar TIA syndromes are caused by the involvement of the penetrating lenticulostriate arteries from the MCA. In our study, the etiology of the TIAs in the non-AF group included not only artery to artery embolism from carotid disease but also the involvement of lenticulostriate branches from the MCA, which often results in hemiparesis without major hemispheric symptoms. This would explain why the non-AF group had hemiparesis without aphasia more frequently than the AF group.

Harrison and Marshall reported that the duration of symptoms in TIA patients with AF tended to be longer than 60 minutes (10). In this study, 50% of the patients with AF had a TIA duration of more than 60 minutes. In 5 of 6 aphasic patients with AF, the TIAs lasted for over 60 minutes. Therefore, patients in AF with a TIA, who present with aphasia are likely to have a TIA of long duration.

Although internal carotid artery dissection is recognized as a cause of TIA (11), our study did not have any patients with internal carotid artery dissection. This is likely because in Japan TIAs and strokes due to internal carotid artery dissection are very rare (12).

Recently, the importance of other emboligenic factors that cause cerebral embolism has been recognized (13). Of particular interest these include: aortic arch atheromatous plaques, patent foramen ovale (PFO), and atrial septal aneurysms. Pop et al studied 72 consecutive TIA patients using

both transthoracic echocardiography (TTE) and transesophageal echocardiography (TEE). They concluded that TEE significantly increased the yield in visualizing potential intracardiac sources of emboli compared with TTE (14). In the present study, patients were not routinely evaluated by TEE, and thus these intracardiac lesions may have been overlooked. However, it was our intention to study the differences between patients with AF and without AF.

Thus, in conclusion, TIA patients with AF appear to be more likely than those without AF to have a major hemispheric syndrome such as aphasia.

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Dissecting aneurysms of the vertebral artery: a management strategy

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Object. The authors present a retrospective analysis of their experience in the treatment of vertebral artery (VA) dissecting aneurysms and propose a management strategy for such aneurysms, with special emphasis on the most formidable VA dissecting aneurysms, which involve the origin of the posterior inferior cerebellar artery (PICA).

Methods. Since 1998, 18 patients with VA dissecting aneurysms, 11 of whom presented with subarachnoid hemorrhage (SAH), have been treated by endovascular surgery at the authors' institution. Obliteration of the entire segment of the dissected site with coils (internal trapping) was performed for aneurysms without involvement of the origin of the PICA (12 cases; among these the treatment-related morbidity rate was 16.7%). The treatment strategy applied to PICA-involved VA dissecting aneurysms presenting with SAH (three cases) included proximal occlusion of the parent artery followed by internal trapping of the aneurysm (one case), proximal occlusion of the parent artery followed by occipital artery (OA)–PICA bypass (one case), and two-staged internal trapping of the aneurysm involving double PICAs (one case). For PICA-involved VA dissecting aneurysms that were not associated with SAH at presentation (three cases), OA–PICA bypass was performed and followed by internal trapping of the aneurysm (two cases). In the remaining case in which a fetal-type posterior communicating artery was present, internal trapping was performed following successful balloon test occlusion (BTO). Overall, there was no sign of infarction in the PICA territory, despite complete occlusion of aneurysms involving the PICA. There was no recurrent bleeding or ischemic symptoms during the follow-up periods. The overall treatment-related morbidity rate for the VA dissecting aneurysms involving the PICA was 16.7%.

Conclusions. Dissecting VA aneurysms that do not involve the PICA can be safely treated by internal trapping. For those lesions that do involve the PICA, a decision-making algorithm is advocated to maximize the efficacy of the treatment as well as to minimize the risks of treatment-related morbidity based on BTO.

KEY WORDS • **dissecting aneurysm** • **subarachnoid hemorrhage** • **vertebral artery** • **coil embolization** • **bypass procedure** • **balloon test occlusion**

With increased awareness of the disease entity and its angiographic appearance, dissecting aneurysms of the VA have now come to be considered a rather common cause of SAH and brainstem ischemia.[5,12,21,30,31] In cases presenting with SAH, previous studies have reported a high incidence of rebleeding and a high mortality rate at the time of recurrent bleeding,[1,19,30] emphasizing the necessity of early treatment. Originally, proximal clipping of the parent artery[5,34] and trapping of the aneurysms[5] by direct surgical approach were advocated as treatments of choice. Nevertheless, relatively benign natural histories have been reported in cases presenting with ischemic symptoms, and the indications and timings for treatment of non-SAH cases remain controversial.[13,21,35]

Recently, the advent of endovascular surgery changed treatment options for VA dissecting aneurysms, especially during the acute phase following SAH, because this technique enables not only occlusion of the parent artery but also obliteration of the entire segment of the dissected site with coils (internal trapping) more easily in a tight posterior fossa, especially with regard to securing the distal side of the parent artery.[8] Proximal parent artery occlusion is not believed to eliminate completely the risk of rebleeding,[1,19,30] but surgical trapping of the dissected site has been reported to be associated with a high rate of postoperative lower cranial nerve palsy.[12] Therefore, internal trapping of the aneurysm by endovascular treatment during the acute stage post-SAH might significantly improve outcomes in patients harboring dissecting VA aneurysms, especially those presenting with SAH.[32] Little is known about the safety and long-term efficacy of internal trapping of aneurysms by endovascular treatment in the prevention of rebleeding. In addition, if the PICA is involved in the dissecting segment (the PICA-involved type of VA dissecting lesion), internal trapping could be associated with a significant risk of morbidity if no revascularization of the PICA is achieved. Al-

Abbreviations used in this paper: AICA = anterior inferior cerebellar artery; BTO = balloon test occlusion; GDC = Guglielmi detachable coil; IDC = interlocking detachable coil; MR = magnetic resonance; OA = occipital artery; PICA = posterior inferior cerebellar artery; SAH = subarachnoid hemorrhage; VA = vertebral artery; VB = vertebrobasilar; WFNS = World Federation of Neurosurgical Societies.

TABLE I

*Summary of patients with VA dissecting aneurysms presenting with SAH**

Case No.	Age (yrs), Sex	WFNS SAH Grade	Aneurysm Location†	Day Post-SAH: Re-bleeding	Day Post-SAH: Ad-mission	Treatment: Pro-cedure	Treatment: Day Post-SAH	Morbidity	Treatment-Related Morbidity	GOS Score	mRS Score	Barthel Index (/20)	F/U Angio	F/U
1	52, M	V	prox, rt	2/3	3	ITA	3	lt hemiparesis, dysarthria, & ataxia	occipital infarct	3 (SD)	4	19	16 mos	28 mos
2	60, M	IV	prox, lt	none	0	ITA	0	transient amnesia	none	5 (GR)	1	20	18 mos	20 mos
3	58, F	II	prox, rt	none	0	ITA	0	lt hemiparesis, horizontal gaze palsy, rt Horner syndrome	none	5 (GR)	1	20	12 mos	14 mos
4	51, M	III	prox, rt	none	0	ITA	0	none	none	5 (GR)	0	20	5 mos	13 mos
5	68, M	III	dis, rt	none	0	ITA	0	none	none	5 (GR)	0	20	—	6 mos‡
6	75, M	V	dis, rt	none	0	ITA	0	brain death by primary damage	none	1 (D)	—	—	—	9 days
7	57, M	V	dis, lt	none	98	ITA	112	dysphagia	brainstem infarct	3 (SD)	4	3	—	2 mos
8	64, M	IV	no PICA, rt	2	2	ITA	2	dysarthria, dysphagia, hemiataxia, & recent memory disturbance	none	3 (SD)	4	17	7 days	21 mos
9	54, M	III	involve, lt	1	1	ITA/ITA	1/5	lt CN VII & rt CN III deficits, dysarthria, dysphagia, visual field defect	occipital infarct	5 (GR)	1	20	13 mos	44 mos
10	60, M	IV	involve, rt	none	2	POPA/ BTO/ ITA	2/32	Terson glands	none	5 (GR)	1	20	20 mos	7 mos
11	51, F	V	involve, lt	0	0	POPA/ bypass	0/23	recent memory disturbance	none	5 (GR)	1	20	14 days	3 mos

* Angio = angiography; bypass = OA–PICA bypass; CN = cranial nerve; D = death; dis = distal to PICA; F/U = follow up; GR = good recovery; involve = PICA location involved; ITA = internal trapping of aneurysm; mRS = modified Rankin scale; POPA = proximal occlusion of parent artery; prox = proximal to PICA; SD = severe disability; — = not applicable.

† In relation to PICA.

‡ Died of lung cancer.

though there have been several case series of VA dissecting aneurysms of the PICA-involved type,[20,33] in no previous reports have authors advocated an ideal management strategy by implementing endovascular techniques for this formidable subgroup of aneurysms. In this study we retrospectively reviewed the safety and long-term efficacy of endovascular treatment for VA dissecting aneurysms, with special emphasis on management outcomes of our novel strategy for aneurysms of the PICA-involved type, with or without revascularization of the PICA, based on the results of BTO.

Clinical Material and Methods

Between January 1998 and May 2001, 499 aneurysms were treated at the National Cardiovascular Center. During this period, 18 cases of VA dissecting aneurysms, comprising approximately 3.6% of the total number of aneurysm cases, were treated by endovascular methods (internal trapping of the aneurysm or proximal occlusion of the parent artery) as the first line of therapy. The diagnosis was made based on characteristic features demonstrated on angiography.[23] The location of each aneurysm was classified into one of three groups: proximal to the PICA, involving the PICA, and distal to the PICA. There were six cases of the PICA-involved type, three presenting with SAH and three not associated with hemorrhage. We retrospectively reviewed the clinical and radiological features of these aneurysms and examined the safety and management outcomes of our strategy.

Patient Population

The mean age of the 18 patients (14 men and four women) was 56 years (range 45–75 years). Eleven patients presented with SAH, four with ischemic symptoms, and one with headache; in the remaining two patients the aneurysm was diagnosed incidentally. Of note, in one patient who initially presented with cerebellar infarction, a minor leak later developed during the chronic phase. A previous history of hypertension was noted in six patients.

Endovascular Procedure

The patient was placed in a state of general anesthesia, and received systemic heparinization. A No. 6 French guide catheter or a No. 7 French balloon catheter was placed in the proximal VA. A microcatheter was delivered coaxially, with the tip placed just beyond the distal end of the dissected site, in the case of internal trapping, or just proximal to the aneurysm, in the case of proximal endovascular occlusion, with or without proximal flow control. Guglielmi detachable coils were delivered to the distal portion of the dissected site in the case of internal trapping, or just proximal to the aneurysm in the case of proximal VA occlusion. After this procedure, a pack of IDCs or fiber coils was sequentially delivered to achieve rapid permanent thrombosis.

Sources of Supplies and Equipment

The Envoy No. 6 French guide catheter and the Commodore nondetachable silicone balloon catheter were purchased from Cordis Endovascular Systems (Miami, FL). The Patlive No. 7 French balloon catheter was obtained from Clinical Supply Inc. (Gifu, Japan). The GDCs, IDCs, and fiber coils were acquired from Target Therapeutics–Boston Scientific (Natick, MA).

Results

The management strategy was tailored to the individual case based on the presenting symptoms (SAH group/non-SAH group), angiographic features of the aneurysms (the location of the aneurysm and the dominancy of the involved VA), and the neurological condition of the patient, as described later.

Patients With SAH

Among the 11 patients presenting with SAH (nine men and two women) (Table 1), the aneurysms were located proximal to the PICA in four, involved the PICA in three, and were located distal to the PICA in three patients; the PICA was absent in one patient. At admission, clinical grading based on the WFNS scale was Grade II in one patient, Grade III in three patients, Grade IV in three patients, and Grade V in four patients. Before treatment, five episodes of recurrent bleeding occurred in four patients within 3 days after the initial bleeding. In 10 patients endovascular treatment was performed on the day of admission: six patients were treated on Day 0, one on Day 1, two on Day 2, and one on Day 3 post-SAH. The remaining patient presented with a Grade V SAH and was referred to our department during the chronic stage; that patient underwent endovascular treatment 3 months after the initial hemorrhage.

If the aneurysm was located proximal or distal to the PICA without hypoplasia of the contralateral VA, the lesion was treated with the intention of occluding the dissected site with coils (internal trapping) so that the PICA would be filled from the contralateral or ipsilateral VA, respectively, after embolization.

In the case of aneurysms involving the PICA, the management strategy was tailored to each individual case as described in the following subsections.

Case 9. This 54-year-old man harbored a left VA dissection involving the origin of the PICA. The aneurysmal dilation was located just distal to the origin of the distal branch of the double PICAs. Our initial attempt to obliterate only the aneurysmal dilation and, thus, spare the distal PICA and inadvertent migration of coils into the pseudolumen precluded tight packing of the aneurysm. Follow-up angiography performed 3 days after treatment revealed persistent filling of the aneurysm, with anastomosis between the double PICAs. During a second procedure, the site of dissection and the distal origin of the double PICA were both completely occluded by coil packing, resulting in an asymptomatic small embolism in the right occipital lobe. No new ischemic deficit developed.

Case 10. This 60-year-old man presented with a WFNS Grade IV SAH. Angiography revealed a right VA dissecting aneurysm involving the origin of the PICA (Fig. 1a and Table 1). Proximal VA occlusion was performed on the day of admission with the intention that the aneurysm would fill slowly in a retrograde fashion from the contralateral VA (Fig. 1b). The patient regained consciousness approximately 1 week later. Follow-up angiography was performed 3 weeks post-SAH, revealing enlargement of the aneurysmal dilation, which was filled from the left VA (Fig. 1c). Balloon test occlusion was performed to assess the feasibility of internal trapping by inflating a nondetachable silicone balloon catheter that had been transported via the contralateral VA to the distal side of the aneurysm (Fig. 1d). During balloon occlusion of the distal VA, we could observe on angiography that the AICA supplied the territory of the PICA via leptomeningeal anastomosis. After the patient tolerated a 15-minute occlusion, GDCs were deployed through the microcatheter, resulting in internal trapping of the aneurysm involving the origin of the PICA without any neurological sequela (Fig. 1e).

Case 11. This 51-year-old woman presented with a WFNS Grade V SAH. A left VA dissecting aneurysm was discovered just proximal to the PICA and extending close to the VB junction (Fig. 2a and Table 1). On Day 0 post-SAH, proximal occlusion of the left VA was performed so that the well-developed PICA could be filled from the contralateral VA (Fig. 2b). Follow-up angiography revealed slight enlargement of the aneurysmal dilation as it filled from the contralateral VA (Fig. 2c). Because the acute angle formed by the bilateral VAs at the VB junction seemed to preclude safe and stable placement of a balloon catheter, we performed an OA–PICA bypass with clipping of the PICA, but no BTO on Day 23 (Fig. 2d). Follow-up angiography performed 14 days later revealed complete thrombosis of the remaining dissecting aneurysm distal to the PICA, without additional deficit (Fig. 2e).

Management Outcomes in the SAH Group. Complete obliteration of the aneurysm was obtained in all cases without any treatment-related death. After our strategy for PICA-involved VA dissecting aneurysms had been applied, diffusion-weighted MR imaging demonstrated no treatment-related, ischemic lesions in the PICA territory. Among the 11 patients who presented with SAH, the final outcomes 6 months posthemorrhage were good recovery in seven patients, severe disability in three patients, and death in one patient (Table 1). The grade of each patient at presentation had a significant impact on ultimate outcome. All patients who presented with WFNS Grades I through III made good recoveries, whereas 67% with Grade IV and 25% with Grade V achieved good recoveries. No symptomatic vasospasm was noted in this series. Factors accounting for poor outcome included primary brain damage in three cases and rebleeding in one case, respectively. At the mean follow-up period of 15.8 months, there were no subsequent ruptures. Follow-up angiograms or MR angiograms were obtained after discharge in six cases at a mean interval of 14 months; no recanalization of aneurysms was observed at that time.

Patients Without SAH

The indication for treatment was limited to cases with progressive enlargement of aneurysmal dilation and recurrent ischemic symptoms. Among the non-SAH group, the aneurysms were located proximal to the PICA in one patient, involving the PICA in three patients, and distal to the PICA in three patients. The management strategy followed

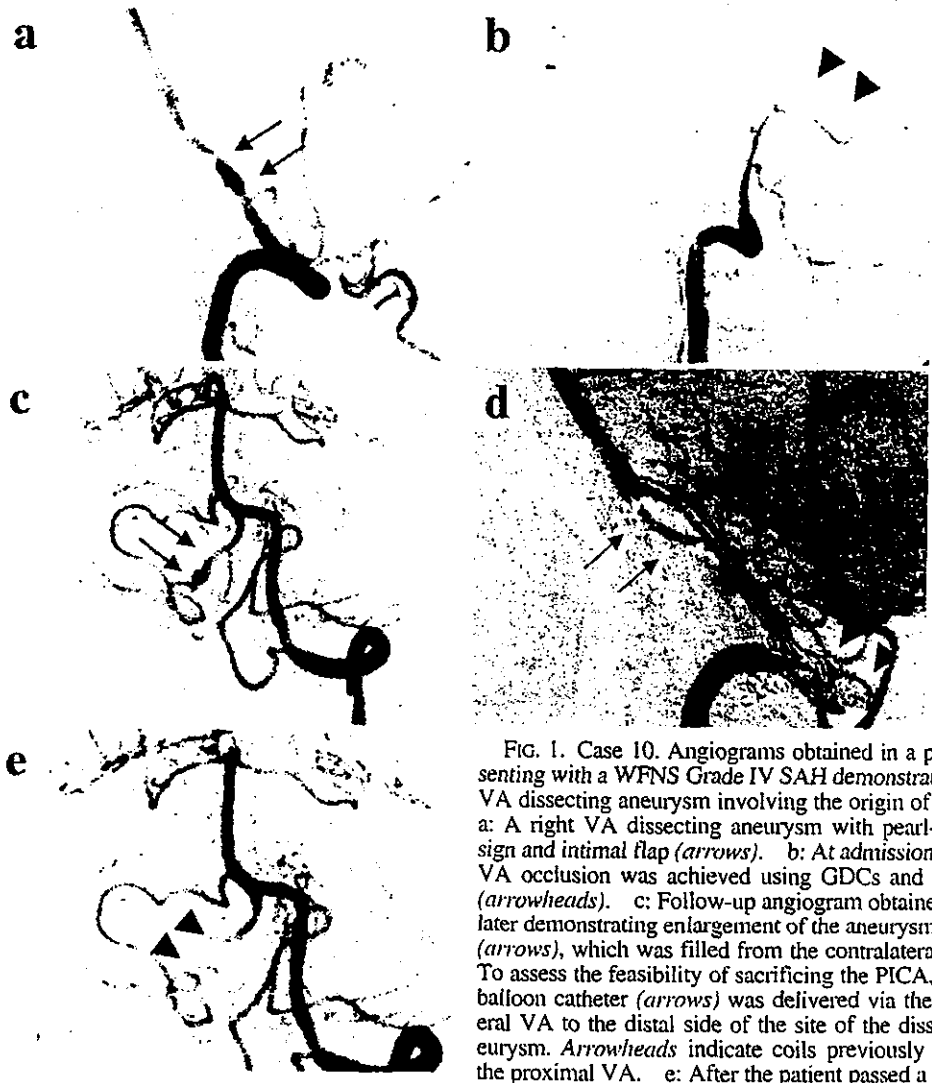

FIG. 1. Case 10. Angiograms obtained in a patient presenting with a WFNS Grade IV SAH demonstrating a right VA dissecting aneurysm involving the origin of the PICA. a: A right VA dissecting aneurysm with pearl-and-string sign and intimal flap *(arrows)*. b: At admission, proximal VA occlusion was achieved using GDCs and fiber coils *(arrowheads)*. c: Follow-up angiogram obtained 3 weeks later demonstrating enlargement of the aneurysmal dilation *(arrows)*, which was filled from the contralateral VA. d: To assess the feasibility of sacrificing the PICA, a silicone balloon catheter *(arrows)* was delivered via the contralateral VA to the distal side of the site of the dissecting aneurysm. *Arrowheads* indicate coils previously packed in the proximal VA. e: After the patient passed a 15-minute BTO, the site of dissection was occluded (internal trapping) by GDC *(arrowheads)* and fiber coils without any sequela. The PICA territory was supplied by leptomeningeal anastomosis from the ipsilateral AICA. Again, *arrowheads* indicate coils previously packed in the proximal VA.

for aneurysms located proximal or distal to the origin of the PICA was virtually the same as that described earlier for the SAH group. The management strategy for aneurysms involving the origin of the PICA again was tailored to the individual case, as in the SAH group.

Case 17. This 54-year-old man presented with a right AICA syndrome caused by brainstem infarction. The aneurysmal dissection was located on the PICA and involved the artery's origin from the VA. Follow-up angiography performed 9 months later revealed enlargement of the aneurysmal dilation. To determine the feasibility of internal trapping of a dissected segment involving the origin of the PICA, BTO was performed. When the balloon was inflated, the patient complained of vertigo and tinnitus associated with documented nystagmus. Because the patient did not pass the BTO, an OA–PICA surgical anastomosis was performed on the right side. After the patient passed a second BTO, coil embolization of the site of dissection (internal trapping) was performed. Follow-up angiography revealed complete obliteration of the aneurysm. The PICA territory was filled from the bypass and there was no evidence of a new ischemic lesion on diffusion-weighted MR images.

Case 18. This 43-year-old man presented with vertigo due to cerebellar infarction. At another hospital the patient had received a diagnosis of dissections at the bilateral VAs with the pearl-and-string sign (Fig. 3 and Table 2). Sixteen days later, a spinal tap revealed xanthochromic CSF, indicating the occurrence of a previous SAH. Angiograms obtained 11 days after the spinal tap demonstrated thrombosis of the left VA and enlargement of the aneurysmal dilation on the right side (Fig. 3a). The bilateral VAs ended in the PICAs and there was retrograde filling of the basilar artery from the bilateral internal carotid arteries via fetal-type posterior communicating arteries (Fig. 3b). The patient underwent a BTO during which a silicone balloon was placed within the right VA and expanded for 20 minutes (Fig. 3c). Following the successful BTO, the aneurysm was occluded with a pack of GDCs and fiber coils (Fig. 3d). No se-

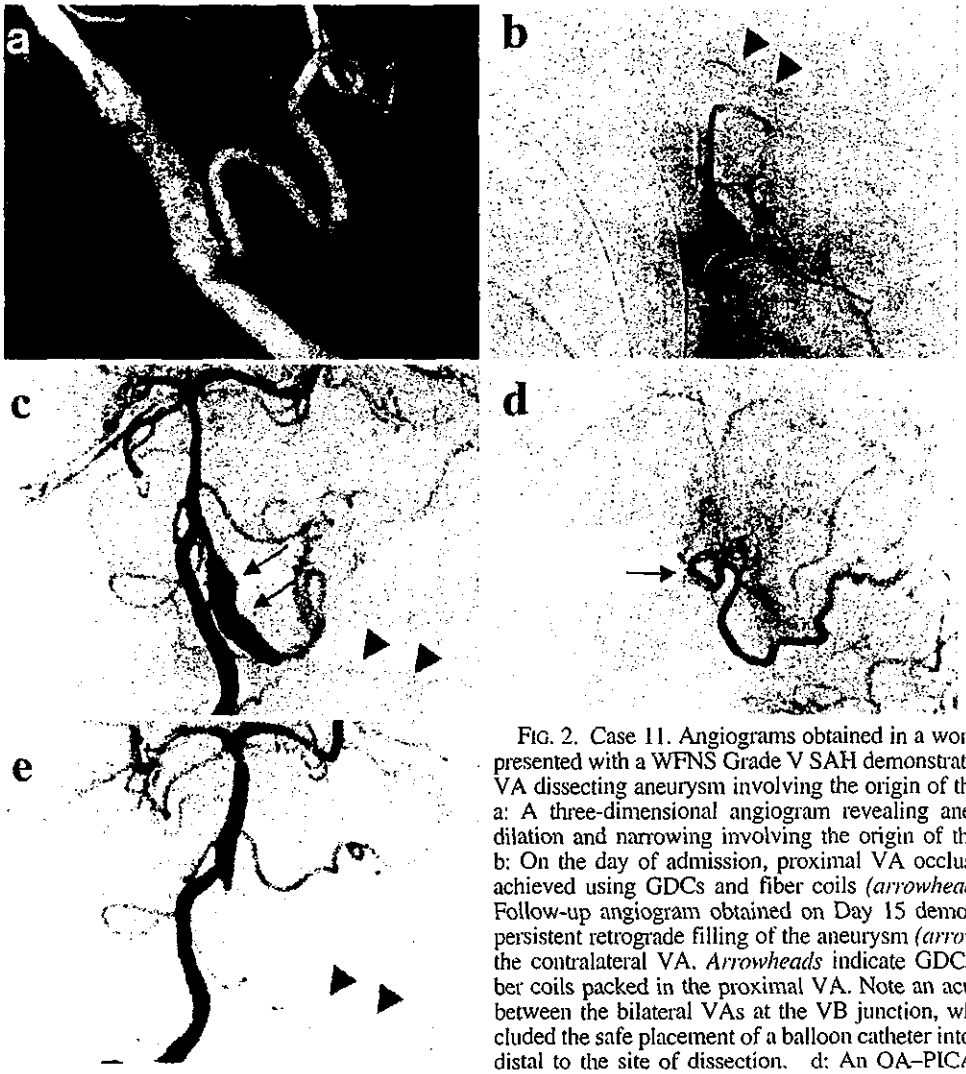

FIG. 2. Case 11. Angiograms obtained in a woman who presented with a WFNS Grade V SAH demonstrating a left VA dissecting aneurysm involving the origin of the PICA. a: A three-dimensional angiogram revealing aneurysmal dilation and narrowing involving the origin of the PICA. b: On the day of admission, proximal VA occlusion was achieved using GDCs and fiber coils *(arrowheads)*. c: Follow-up angiogram obtained on Day 15 demonstrating persistent retrograde filling of the aneurysm *(arrows)* from the contralateral VA. *Arrowheads* indicate GDCs and fiber coils packed in the proximal VA. Note an acute angle between the bilateral VAs at the VB junction, which precluded the safe placement of a balloon catheter into the side distal to the site of dissection. d: An OA–PICA bypass *(arrow)* has been created and the origin of the PICA has been clipped. e: Complete thrombosis of the site of dissection is seen on a follow-up angiogram obtained on Day 38. *Arrowheads* again indicate GDCs and fiber coils packed in the proximal VA.

quela occurred. Bilaterally, the PICA territories were supplied from the leptomeningeal anastomosis via the bilateral AICAs.

Management Outcomes in the Non-SAH Group. Complete obliteration of the aneurysm was obtained in all cases without any treatment-related death. There was a 0% permanent treatment-related morbidity rate at 6 months postoperatively and there was only one case of transient amnesia (Case 12 [Table 2]) in the non-SAH group. Follow-up angiography was performed after discharge in four cases, and no recanalization was revealed at the mean follow-up period of 9.3 months. No recurrent ischemic symptoms or conversions to hemorrhage were seen following treatment.

Safety of the Endovascular Procedure

No major bleeding complications occurred during placement of coils in the affected VAs. Minor extravasation during coil placement and rebleeding during placement of the guide catheter were seen in one case each and were controlled by delivery of coils to the dissected site.

With our management strategy for the PICA-involved type (six cases), there was one case (16.7%) of treatment-related morbidity (occipital infarction due to embolism in Case 9 [Table 1]). There was no new lesion in the PICA territory on diffusion-weighted MR imaging posttreatment. The morbidity rate for PICA-involved VA dissecting aneurysms was the same as that for VA dissecting aneurysms without any PICA involvement.

Discussion

In this article we reported on the safety of endovascular treatment for VA dissecting aneurysms and the long-term efficacy of prevention of recurrent bleeding after SAH. In line with previous reports, our results of endovascular treatment for VA dissecting aneurysms located proximal or distal to the PICA were satisfactory, with an acceptable,

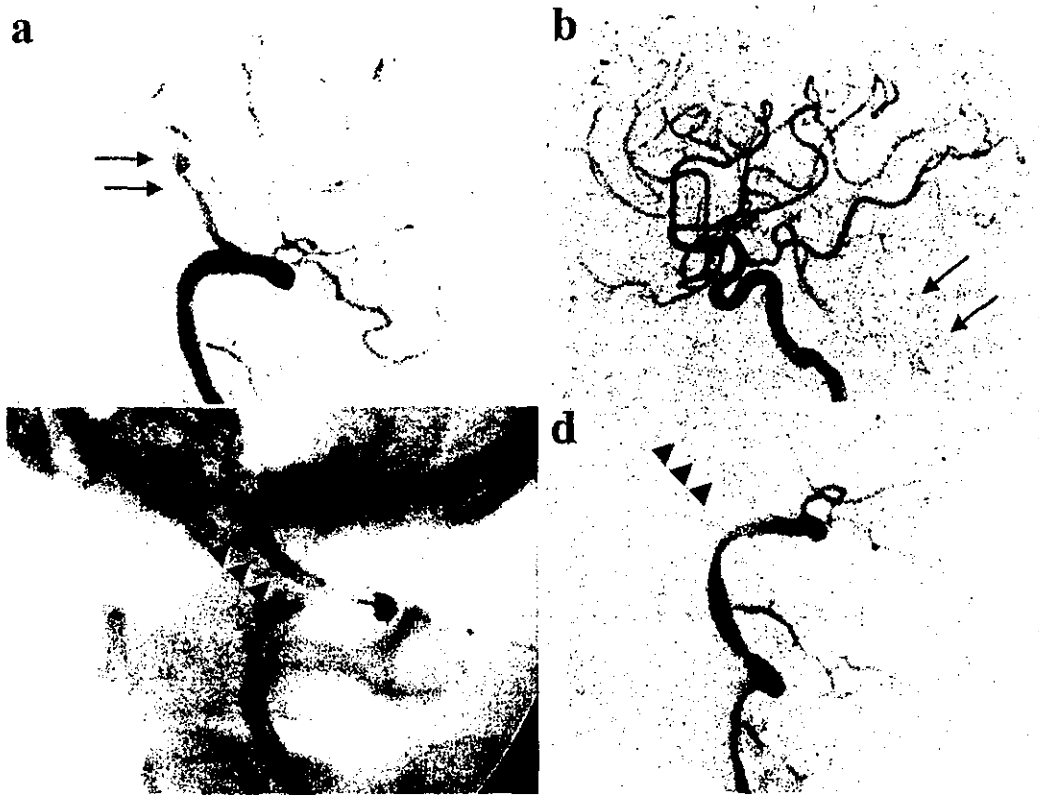

FIG. 3. Case 18. Angiograms obtained in a patient with a VA dissecting aneurysm involving the PICA who presented with a cerebellar infarction. The angiograms demonstrate bilateral dissecting aneurysms with the pearl-and-string sign. a: Follow-up angiogram showed thrombosis of the left VA and an aneurysmal dilation on the right VA involving the PICA *(arrows)*. b: Carotid angiogram demonstrating retrograde filling of the basilar artery and bilateral PICA territories supplied via fetal-type posterior communicating arteries. c: The patient tolerated a 20-minute BTO of the right VA. *Arrowheads* indicate nondetachable balloon placed in proximal VA. d: Angiogram confirming that the aneurysm is occluded with GDCs and fiber coils *(arrowheads)* packed in the VA.

treatment-related rate of morbidity and no treatment-related death.[14,17,18,32] Unfortunately, there is no established protocol for the management of VA dissecting aneurysms of the PICA-involved type. We assessed the feasibility of sacrifice of the PICA by applying a BTO when possible in such cases during the chronic stage after SAH, and sacrificed the PICA with or without revascularization, depending on the result of this test, without any neurological sequela.

Indications and Optimal Timing of the Treatment

It is known that VA dissecting aneurysms that present with SAH are associated with a high risk of rebleeding, especially within the first 24 hours posthemorrhage and within the 1st week if left untreated.[1,19,30] Therapeutic intervention should thus be performed as soon as possible to prevent subsequent bleeding. In addition, because a VA dissecting aneurysm has been reported to rebleed more than 1 month after the initial episode,[19] during the chronic stage following SAH aneurysms could also be targets for therapeutic intervention. On the other hand, the therapeutic indications for VA dissecting aneurysms presenting with signs and symptoms other than SAH, such as VB ischemia, headache, and incidental findings remain an open question. Conservative treatment has been recommended for the non-SAH group because a relatively benign clinical course and outcome have been reported for these aneurysms.[13,21,35] A recent study, however, demonstrated serial angiographic changes in 88.2% of unruptured VA dissections, including subsequent formations of aneurysms that may be amenable to surgical treatment,[22] as seen in Case 17 in the present series. In fact, another recent report has shown that VA dissections of the ischemic type have developed into subsequent SAH at a rate of 3.4%.[31] Therefore, we recommend follow-up angiography for VA dissections in non-SAH cases during the early stage (< 3 weeks) after presentation and, if subsequent formation of aneurysms is documented, therapeutic intervention is necessary to prevent bleeding.

Mode of Treatment: Endovascular or Surgical Treatment

Originally, proximal clip occlusion or trapping was advocated as the treatment of choice for VA dissecting aneurysms if the contralateral VA is equal or greater in caliber, and wrapping of the dissection if the affected artery is dominant.[30] Using BTO, Halbach, et al.,[8] have shown that patients can tolerate permanent occlusion of the dominant VA and, even, bilateral VA occlusion without deficits. Because the dissecting aneurysms are extremely fragile and easily ruptured during surgery, endovascular proximal occlusion of the parent vessel seems to be the safest method, especially during the acute stage following SAH. In recent papers, however, authors have shown that proximal occlusion achieved by endovascular means as well as by clipping

TABLE 2

*Summary of patients with VA dissecting aneurysms presenting with non-SAH symptoms**

Case No.	Age (yrs), Sex	Presentation	Aneurysm Location†	Treatment: Procedure	Treatment: Timing After Symptom Onset	Morbidity	Treatment-Related Morbidity	GOS Score	mRS Score	Barthel Index (/20)	F/U Angio (mos)	F/U (mos)
12	49, M	incidental	prox, rt	ITA	—	none	transient amnesia	5 (GR)	0	20	7	22
13	55, F	ischemia	dis, rt	ITA	11 mos (recanalization)	none	none	5 (GR)	1	20	8	28
14	50, M	ischemia	dis, rt	ITA	8 mos	none	none	5 (GR)	0	20	—	27
15	47, F	HA	dis, lt	ITA	2 mos	none	none	5 (GR)	1	20	—	6
16	45, M	incidental	involve, lt	bypass/ITA	none	none	none	5 (GR)	0	20	15	35
17	54, M	ischemia (rt CN VII & CN VIII deficits)	involve, rt	BTO/bypass/BTO/ITA	10 mos	rt CN VII & CN VIII deficits	none	5 (GR)	1	20	7	24
18	43, M	ischemia (rt cerebellum)	involve, rt	BTO/ITA	1 mo	none	none	5 (GR)	1	20	—	9

* HA = headache.
† In relation to PICA.

does not completely eliminate the risk of rebleeding from unclippable VA aneurysms.[1,7,11,30] Therefore, internal trapping of the dissected site is now deemed as the best treatment of choice, if feasible, for VA dissecting aneurysms.[32] There are several important technical aspects of internal trapping of the dissecting aneurysms. The GDC system allows controlled placement and detachment of soft platinum coils within the true lumen of the dissected segment to collapse the false lumen.[32] Appropriate selection of the GDCs that are first used, and the subsequent use of fiber coils and IDCs enables safe and rapid occlusion of the affected segment. In addition, temporary proximal flow arrest, achieved using a nondetachable balloon, may reduce the risk of distal emboli and preclude difficulties in the dispersal of coils into the arterial flow stream.[6] Also, it is important to prevent the coils from becoming inadvertently packed into the pseudolumen, which would make it not only dangerous but also difficult to pack the aneurysm tightly.

Management Strategy for VA Dissecting Aneurysms Involving the PICA

Dissecting aneurysms of the VA that involve the PICA represent the most formidable subgroup. The therapeutic goal for a VA dissecting aneurysm must be complete exclusion of the lesion from the circulation. As long as even the slightest filling is present, the patient remains at risk for hemorrhage. According to a recent case report,[33] there have been only 10 cases of PICA-involved VA dissecting aneurysms, five of which were treated with revascularization of the PICA. Therapeutic options reported thus far have included proximal clipping of the VA (three cases),[5,11] endovascular proximal occlusion of the VA (two cases),[29,33] and trapping of the dissected segment with (three cases)[4,10,27] or without (two cases)[2,33] revascularization of the PICA (OA–PICA, or PICA–PICA anastomosis). In previous series the overall morbidity rate in the management of PICA-involved VA dissecting aneurysms was as high as 40%.[33] The morbidity rate was much higher in the subgroup treated by trapping (60%) than in the subgroup that underwent proximal occlusion of the VA (20%). In fact, clipping of the proximal PICA combined with trapping of the aneurysm resulted in cerebellar and brainstem infarction.[2] Because the portion of the PICA proximal to the choroidal point (the site at which the PICA produces small rami to the anterior aspect of the tonsil and the choroid plexus of the fourth ventricle) may produce critical perforating arteries to the brainstem and deep cerebellar nuclei,[15] it is hard to predict whether it is safe to trap an aneurysm involving the origin of the PICA without revascularization. On the other hand, in previous cases proximal clip placement or endovascular proximal occlusion of the VA led to later reoperation for trapping of the dissected segment in two (40%) of the five cases, either because a repeated rupture occurred (one case)[11] or because enlargement of the aneurysms was detected at serial angiograms (one case).[33] Although it is possible to perform revascularization of the PICA and trapping of the aneurysm during a single operation to eliminate the risk of rebleeding, this is technically much more demanding, especially during the acute stage post-SAH. To overcome the dilemma this raises, we advocate use of a decision-making algorithm for the treatment for the VA dissecting aneurysms of the PICA-involved type (Fig. 4).

As soon as the diagnosis of dissecting aneurysm involving the PICA is established, endovascular proximal occlusion of the VA should be performed using a pack of GDCs and fiber coils, as described earlier. If the other intact VA is hypoplastic or absent, BTO of the affected parent VA should be performed under neurophysiological monitoring to determine whether it has been safely performed, as described previously.[25] After the patient has survived the acute stage of SAH with improvement in consciousness, follow-up angiography is performed to examine whether the aneurysm continues to be filled from the contralateral VA. Progressive thrombosis may occur in the segment distal to the PICA because the small perforating vessels or a very small PICA provides an inadequate supply to keep this segment patent from retrograde flow from the basilar artery. If the aneurysm continues to fill from the contralateral VA, a nondetachable silicone balloon catheter is delivered via the contralateral VA, placed just distal to the dissected site, and inflated for 20 minutes to determine whether the dissect-

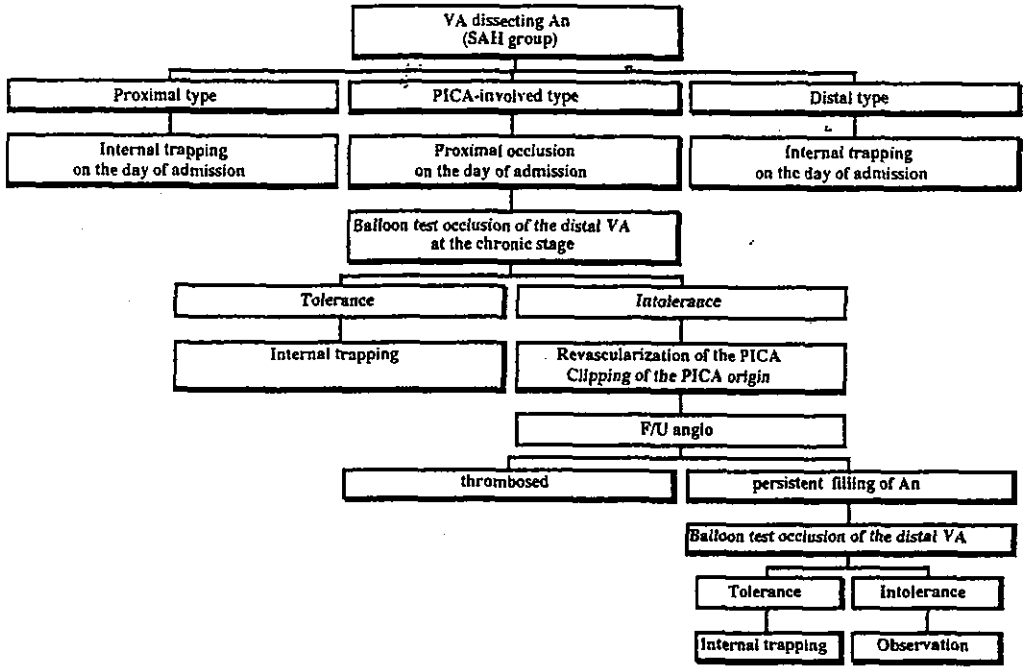

FIG. 4. Proposed management strategy for VA dissecting aneurysms. An = aneurysm.

ed site can be safely trapped with coils (internal trapping). During BTO, contralateral VA and ipsilateral external carotid arteriography are performed to examine whether the PICA territory is supplied from collateral routes such as leptomeningeal anastomoses from the AICA and superior cerebellar artery or anastomoses from the posterior meningeal artery. If the patient tolerates a 20-minute occlusion with angiographic demonstration of filling of the PICA territory from collateral routes, and if no neurological manifestation of ischemia in the PICA territory (such as vertigo, tinnitus, and nystagmus [see Case 17]) is present, internal trapping of the dissected site can be performed. In the case of an unsuccessful test occlusion of the distal end of the dissected site, the PICA should be revascularized by means of an OA–PICA[9,26] or a PICA–PICA[27] bypass and the origin of the PICA should be clipped. At this stage, the dissected site will likely become thrombosed because this segment between the PICA and the VB junction becomes a blind sac, with no sufficient demand to keep the segment open. Of note, in our series, there was no treatment-related infarction in the PICA territory associated with our strategy for treating the PICA-involved type of lesion, and the overall treatment-related morbidity rate was the same as that for other VA dissecting aneurysms.

Unsolved Problems

The safety of internal trapping of the dissected site remains unestablished. At surgery, large perforating arteries are often observed to arise from dissecting aneurysms. Nevertheless, patients tolerate complete thrombosis of the aneurysm by the adjacent segment of the VA without the development of brainstem ischemia because the perforating vessels arising from the dissected segment are more likely to be occluded already. As in our Case 7, however, in which the dissection was located distal to the PICA, internal trapping of the dissected site can be associated with the development of a brainstem infarction. In Case 7, we did not perform a BTO because the dissection was located distal to the PICA. Anatomical studies have shown critical perforating vessels arising from the distal VAs, especially the nondominant, usually right-sided VA,[16] and also arising from VB arteries between approximately 14 mm proximal and 16 mm distal to the VB junction. In line with these findings, complications in the series conducted by Yamaura occurred in all three patients who underwent clip occlusion of a dissecting aneurysm arising from the vertebral segment above the origin of the PICA.[30] Therefore, it seems more critical to determine when the distal side of the dissection should be occluded and at what particular site. So far, there has been no reliable way to predict the safety of trapping of the dissected site involving the origin of the PICA, although some angiographic criteria have been proposed.[30] We consider the best and most realistic method for this purpose to be BTO in which the nondetachable balloon is placed at the distal side of the dissection at the earliest opportunity after the patient regains consciousness.

We must admit that there remain unsolved problems in our strategy. First, the patient continues to be subject to the risk of rebleeding until internal trapping is performed. In this context, it is important to note that VA occlusion proximal to the PICA carries a greater risk than occlusion distal to the PICA in the treatment of unclippable VA aneurysms,[28] although this is inconsistent with findings of other series.[3,24] Second, the patient has to have recovered consciousness to determine the safety of the test occlusion, although a possible alternative is to use neurophysiological monitoring. Because the reliability of neurophysiological monitoring during the BTO of the PICA remains unknown, we waited until the patient regained consciousness after SAH in this series. It is apparent that more clinical experience is necessary to answer all these questions. Our

preliminary experience, however, has indicated that our management strategy for VA dissecting aneurysms that involve the PICA is close to optimal in safety as well as in intermediate-term efficacy of prevention of bleeding after devastating SAH.

Conclusions

The present case series provided us with a unique opportunity to consider the optimal management strategy for VA dissecting aneurysms, especially the most formidable lesions involving the PICA. We have described our successful treatment of this type of aneurysm, with an acceptable rate of morbidity, by proximal endovascular occlusion performed during the acute stage followed by internal trapping of the dissected site, if possible, based on the results of a BTO of the distal VA segment of the dissected site. Based on our preliminary experience, we advocate a decision-making algorithm for this subgroup of VA dissecting aneurysms.

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Lp(a) Particles Mold Fibrin-Binding Properties of Apo(a) in Size-Dependent Manner

A Study With Different-Length Recombinant Apo(a), Native Lp(a), and Monoclonal Antibody

Chantal Kang,* Miguel Dominguez,* Stéphane Loyau, Toshiyuki Miyata, Vincent Durlach, Edouard Anglés-Cano

Objective—Small-sized apolipoprotein(a) [apo(a)] isoforms with high antifibrinolytic activity are frequently found in cardiovascular diseases, suggesting a role for apo(a) size in atherothrombosis. To test this hypothesis, we sought to characterize the lysine (fibrin)-binding function of isolated apo(a) of variable sizes.

Methods and Results—Recombinant apo(a) [r-apo(a)] preparations consisting of 10 to 34 kringles and a monoclonal antibody that neutralizes the lysine-binding function were produced and used in parallel with lipoprotein(a) [Lp(a)] particles isolated from plasma in fibrin-binding studies. All r-apo(a) preparations displayed similar affinity and specificity for lysine residues on fibrin regardless of size (K_d 3.6±0.3 nmol/L) and inhibited the binding of plasminogen with a similar intensity (IC_{50} 16.8±5.4 nmol/L). In contrast, native Lp(a) particles displayed fibrin affinities that were in inverse relationship with the apo(a) kringle number. Thus, a 15-kringle apo(a) separated from Lp(a) and a 34-kringle r-apo(a) displayed an affinity for fibrin that was higher than that in the corresponding particles (K_d 2.5 versus 10.5 nmol/L and K_d 3.8 versus 541 nmol/L, respectively). However, fibrin-binding specificity of the r-apo(a) preparations and the Lp(a) particles was efficiently neutralized (IC_{50} 0.07 and 4 nmol/L) by a monoclonal antibody directed against the lysine-binding function of kringle IV-10.

Conclusions—Our data indicate that fibrin binding is an intrinsic property of apo(a) modulated by the composite structure of the Lp(a) particle. ***(Arterioscler Thromb Vasc Biol. 2002;22:1232-1238.)***

Key Words: lipoprotein(a) ■ apolipoprotein(a) ■ plasminogen ■ fibrin surfaces ■ fibrin affinity

High plasma levels of the Lp(a) are now recognized as a risk factor in cerebrovascular and cardiovascular diseases.[1] However, the mechanism by which Lp(a) may favor the atherogenic and thrombogenic processes is not, as yet, clearly understood. Its composite structure, consisting of an inactive serine proteinase, apo(a), which is disulfide-linked to apoB-100 of an atherogenic LDL-like particle,[2] may explain this phenomenon in part. Apo(a) is a highly glycosylated protein that shares a high degree of sequence identity (75% to 94%) with plasminogen, the precursor of the fibrinolytic enzyme plasmin.[3,4] Apo(a) and plasminogen are derived from a common ancestral gene and are constituted by disulfide-bridged structures of 80 to 90 amino acid residues called kringle (K) domains and a catalytic domain. Plasminogen consists of 5 different kringle domains, 2 of which, K1 and K4, bear a lysine-binding site (LBS) that displays affinity for lysine residues exposed on fibrin and cell membrane proteins. Binding and activation of plasminogen through these interactions leads to fibrinolysis and pericellular proteolysis. Apo(a) contains a copy of plasminogen-like K5 and multiple tandem repeats of a kringle that shares 61% to 75% sequence identity with K4, hereafter designated K-IV. The K-IV repeats of apo(a) differ by specific point mutations or deletions and have been classified as 10 different types.[5] Apo(a) K-IV type 2 has no functional LBS, and its variable number gives rise to a series of apo(a) isoforms containing a single copy of the other 9 K-IV types.[6] Sequence comparison and molecular modeling[5,7] have shown that an LBS similar to that of plasminogen K4 is present in K-IV type 10. A slightly modified LBS that is present in K-IV copies (type 5 to type 8) and appears to be masked in the intact Lp(a) particle may display lysine (fibrin) binding.[8,9] Kringle copies with a functional LBS may endow apo(a) with lysine-binding capabilities similar to those of plasminogen. Indeed, it has been

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recently shown that apo(a) accumulation in the vessel wall of fibrin(ogen)-deficient apo(a) transgenic mice is importantly reduced.[10] Lp(a) particles containing distinct apo(a) isoforms may show variability in their lysine-binding function[11-13] and display functional heterogeneity for fibrin[14,15] and cell[16] binding. Furthermore, several studies have found a correlation between the prevalence of low-molecular-mass isoforms and coronary artery disease[17,18] or advanced stenotic atherosclerosis.[19] In the present study, we have further explored the existence of such a structural/functional relationship in the interaction of apo(a) with fibrin by using recombinant apo(a) [r-apo(a)] isoforms containing a variable number of K-IV type 2 repeats and a monoclonal antibody (mAb) that neutralizes the LBS function.[20] The results of the present study show that in contrast to native Lp(a) particles, all r-apo(a)s display similar affinity and specificity for lysine residues regardless of size, thus suggesting that the affinity of apo(a) for fibrin is conditioned by the composite structure of the Lp(a) particle.

Methods

Production of r-Apo(a)

The plasmids pCMV-A10, -A14, -A18, -A22, -A26, -A30, and -A34 were obtained as previously described[21] and were stably transfected by electroporation into the adenovirus-transformed human embryonic kidney cell line 293.[22] The culture medium containing the different r-apo(a)s, produced and harvested as described,[23] was supplemented with proteinase inhibitors (20 kallikrein inhibitory units/mL aprotinin, 0.5 mmol/L AEBSF, 2 mmol/L EDTA, and 0.01% [wt/vol] NaN_3, final concentrations) and stored at −80°C until use.

Purification and Amino-Terminal Sequence Analysis of r-Apo(a)

The r-apo(a)s were immunopurified on a Sepharose 4B–immobilized mAb directed against native apo(a) (mAb a3, kindly provided by L. Sorell, Center for Genetic Engineering, Havana, Cuba).[24] The r-apo(a) was eluted with 20 mmol/L glycine-HCl, pH 2.0, containing 0.1 mol/L NaCl, and fractions were collected into a volume of 0.1 mmol/L Tris, pH 10, sufficient to neutralize the pH in each fraction. Fractions containing the r-apo(a) were pooled, concentrated on dried polyethylene glycol 20 000 (Serva), and dialyzed against buffer A (50 mmol/L sodium phosphate, pH 7.4, 80 mmol/L NaCl, 0.01% [wt/vol] Tween 20, and 0.01% [wt/vol] NaN_3) containing 2 mmol/L EDTA. The concentration of the r-apo(a) was determined according to the method of Lowry et al,[25] with purified human plasminogen used as a standard. Electrophoretic analysis (SDS/6% acrylamide or 3.75% polyacrylamide/0.8% agarose gels) of the r-apo(a) preparations was performed as previously described.[15,23] Proteins were electroblotted onto polyvinylidene difluoride sheets with a graphite electroblotter system (Millipore) according to the method of Kyhse-Andersen.[26] Apo(a) protein bands were stained with Coomassie brilliant blue and submitted to microsequencing with the use of a microsequencer (model 473A, Applied Biosystem) equipped with a model 610A data analysis system.

Purification of Native Lp(a) From Plasma and Isolation of Apo(a)

Volunteer blood donors were selected among individuals attending the outpatient clinic and from the medical staff of Hôpital Robert-Debré (Université de Reims, Reims, France) for their high Lp(a) plasma concentration (≥0.5 g/L) and apo(a) phenotype. Venous blood was drawn from the forearm into sterile polypropylene tubes containing 4 mmol/L EDTA (final concentration). Plasma was separated by centrifugation at 2000g for 20 minutes at 4°C and supplemented with proteinase inhibitors and an antioxidant (butyl hydroxytoluene, 0.05 mg/mL). Lp(a) was purified by sequential ultracentrifugation and molecular sieving as previously described.[16] Dissociation of apo(a) 15 kringles from the purified Lp(a) of a homozygous subject [plasma Lp(a), 1.2 g/L] was performed under mild reductive conditions essentially as described by Edelstein et al.[27] After reduction, the solution was adjusted to a solvent density of 1.150 g/mL with KBr and centrifuged at 134 000g for 24 hours at 10°C in a 50-Ti rotor (Beckman). The bottom fraction (extensively dialyzed against buffer A) was shown to contain free apo(a) by SDS-PAGE and Western blotting and was conserved at 4°C until assay (<24 hours).

Purification and Radioiodination of Proteins

Plasminogen was purified from fresh-frozen human plasma as previously described,[28] with modifications.[29] The plasminogen preparation was considered to be >99% pure and was shown to be Lp(a) free, as assessed by SDS-PAGE and autoradiography of the ^{125}I-labeled product and by amino terminal analysis. Plasminogen concentration was calculated by measuring the absorbance at 280 nm with a molecular weight of 93 000 and $\varepsilon^{1\%}_{280\,nm,\,1\,cm}$=16.8[30].

A sheep antiserum directed against human apo(a) was prepared at the Institut National de la Recherche Agronomique (Center de Clermont-Ferrand-Theix) by immunizing the animal with 3 subcutaneous injections of purified r-apo(a) A10. The IgG fraction of this antiserum was separated by ammonium sulfate precipitation, ion-exchange chromatography on diethylaminoethyl-Tris-acryl, and affinity chromatography on protein A–Sepharose (Pharmacia). The purified IgG was further immunodepleted by using Sepharose-immobilized plasminogen, and its apparent affinity for the different r-apo(a) was determined.

The monoclonal IgG1 A10.2 recognizing K-IV type 10 of r-apo(a) was obtained, cultured, and purified on protein A–Sepharose as previously described.[20] Concentration of the purified IgG1 was determined by the procedure of Lowry et al.[25]

Plasminogen and the purified anti-apo(a) IgG were radioiodinated with sodium ^{125}I with the use of the Iodogen method of Fracker and Speck,[31] an iodination time of 4 minutes at 4°C, and the removal of free ^{125}I by molecular sieving on a PD-10 Sephadex column (Pharmacia). The specific radioactivity obtained was 9 to 11 nCi/ng plasminogen and ≈3 nCi/ng anti-apo(a) IgG.

Fibrin-Binding Studies

Fibrin surfaces were prepared and immunocharacterized as previously described.[29] Varying concentrations of purified Lp(a) or r-apo(a) preparations and isolated 15-kringle apo(a) in buffer A supplemented with 40 mg/mL BSA were incubated with fibrin for 2 hours at 22°C. Unbound proteins were removed by 3 washes with buffer A supplemented with 2 mg/mL BSA. The surfaces were then probed with a known concentration of the ^{125}I-labeled polyclonal antibody directed against apo(a) in buffer A supplemented with 40 mg/mL BSA. After 3 washes with buffer A supplemented with 2 mg/mL BSA, bound radioactivity was counted in a γ-radiation counter and transformed into mass of antibody bound by using the specific radioactivity (dpm/mole) of the labeled IgG. Binding of ligands to fibrin in the presence of 0.2 mol/L of 6-amino-hexanoic acid was considered to be unrelated to interactions with lysine residues.

Inhibition of the Fibrin-Binding Function of r-Apo(a) and Lp(a) With a mAb

Experiments aimed at determining the fibrin-binding function of r-apo(a) and plasma Lp(a) were performed with mAb A10.2. This antibody is specifically directed against the LBS of kringle 4 in plasminogen and K-IV type 10 in apo(a)[20] and does not recognize nonfunctional LBSs. Experiments were performed as indicated above except that the r-apo(a) was incubated with varying concentrations of mAb A10.2 or an unrelated mAb (7D4 directed against plasminogen activator inhibitor-1, kindly provided by P.J. Declerck,

Catholic University of Leuven, Leuven, Belgium) for 30 minutes at room temperature before incubation with the fibrin surfaces.

For Lp(a) in plasma, the procedure was as described previously.[32] Briefly, plasma was supplemented with protease inhibitors and incubated with varying concentrations of mAb A10.2. The samples were then incubated (50 μL per well) with fibrin for 1 hour at 37°C; the plates were washed and probed with a polyclonal antibody directed against apo(a). A peroxidase-labeled rabbit anti-sheep IgG was used as secondary antibody, and a 1 mg/mL solution of 2,2'-azinobis(3-ethylbenzthiazoline-sulfonic acid) was used as a substrate for color development. The absorbance was measured in a microplate counter at 405 nm. The amount of r-apo(a) or Lp(a) bound to fibrin was calculated by using the linear regression of the relationship between the concentration of a r-apo(a) reference and the change in absorbance at 405 nm (A_{405}/min).

Competitive Inhibition of Plasminogen Binding to Fibrin

Solutions containing varying amounts (0 to 200 nmol/L) of r-apo(a) and a constant amount (≈4 nmol/L) of radiolabeled plasminogen were prepared in buffer A supplemented with 40 mg/mL BSA and incubated for 2 hours at 22°C with the fibrin surface (50 μL per well). In control experiments, similar amounts of LDL were added to plasminogen and incubated with fibrin. After elimination of the supernatant, the plate was washed, and the radioactivity bound to the surface was counted in a γ-radiation counter. In the absence of competitors, this amount was considered to be 100% plasminogen bound.

Analysis of Binding Data

Original raw data were fitted to the Langmuir adsorption equation for bimolecular interactions at heterogeneous interfaces[29-33]:

$$(1) \qquad (S \cdot X) = S_0 \frac{K[X]}{1+K[X]} = \frac{S_0[X]}{[X]+K^{-1}}$$

where $S \cdot X$ is the equilibrium fraction of r-apo(a) bound to the surfaces, S_0 is the total amount of r-apo(a) binding sites, K is the association constant of the ligand/surface interaction, and X is the total input of ligand. By relating the number of occupied sites [$S \cdot X$=bound anti-apo(a) antibody] to ligand concentration [X=r-apo(a)], Equation 1 allows calculation of the maximum bound, $B_{max}=S_0$, and the dissociation constant, $K_d=K^{-1}$. The amount of r-apo(a) bound to cells or fibrin was expressed by the mass of antibody bound by using an algebraic expression from Equation 1 that describes the linear relationship of antibody binding at low antibody concentrations with the amount of Lp(a) bound to fibrin[34]:

$$(2) \qquad \text{antibody bound} \propto Fn \cdot Lp(a) \times K \times [\text{antibody}]$$

Results

Production, Isolation, and Amino-Terminal Characterization of r-Apo(a) Isoforms

Stable transfected embryonic human kidney cells, line 293, were committed to express r-apo(a) isoforms differing by their variable number of K-IV type 2 repeats (from 0 to 24). All other K-IV types (K-IV type 1 and K-IV type 3 to K-IV type 10) and the serine-protease region were encoded by a common cDNA expression plasmid fragment and were present in single identical copies in all r-apo(a) isoforms (Figure 1). Conditions chosen for development of the transfected cells allowed for the harvesting of culture medium containing high concentrations of intact unmodified r-apo(a) as detected by immunoblot analysis of agarose-acrylamide gels (Figure 2A). The immunopurified r-apo(a) migrated as single protein bands in Coomassie blue–stained 6% acrylamide gels (Figure 2B). The sequence of 10 N-terminal amino acid residues of the purified r-apo(a) was similar to the deduced N-terminal sequence of human apo(a).[3,4]

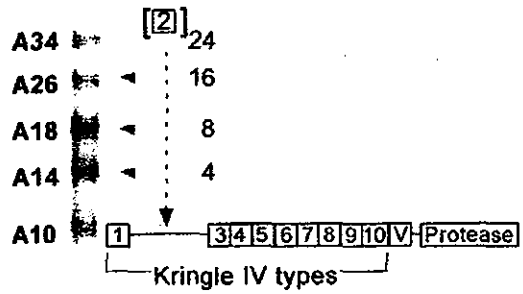

Figure 1. Schematic representation and relative mobility of r-apo(a) isoforms. The domain structure of r-apo(a) isoforms encoded by pCMV-A10 to pCMV-A34 plasmids and relative migration of the expressed proteins (mixture of culture supernatants from transfected human embryo kidney cells) is shown. Electrophoresis was performed on 1.5% agarose gel, followed by immunoblotting, as indicated in Figure 2. Isoform A10 contains single copies of all K-IV types but K-IV type 2. The A14 to A34 isoforms contain a variable number, from 4 (A14) to 24 (A34), of K-IV type 2 copies; all other kringle structures are present in single copies.

Variable Size Apo(a) Recombinants, but Not Lp(a)s, Bind to Fibrin With a Similar Affinity

The use of solid-phase fibrin as a surface acceptor for r-apo(a), native Lp(a), and plasminogen has been previously demonstrated.[15,29,35] Binding isotherms were obtained by

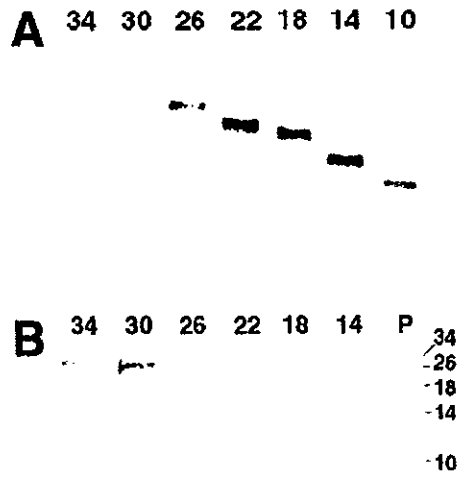

Figure 2. SDS-electrophoresis and immunoblot analysis of purified r-apo(a) isoforms. r-Apo(a) isoforms were purified as described in Methods, reduced with 100 mmol/L dithiothreitol for 30 minutes at 60°C, and electrophoresed in the presence of SDS as indicated. A, r-Apo(a) protein bands were transferred from a 3.75% polyacrylamide/0.8% agarose gel to a nitrocellulose membrane and were immunolocalized with a sheep antibody to r-apo(a) A10, followed by a peroxidase-conjugated rabbit antibody to sheep IgG, revealed with 4-chloro-1-naphthol. A less efficient transfer was observed for isoforms A30 and A34. B, Coomassie brilliant blue staining of r-apo(a) isoforms electrophoresed on a 6% acrylamide gel is shown. Numbers at the top of each figure indicated the total number of kringles of each r-apo(a) isoform. P indicates a pool containing the indicated purified r-apo(a).

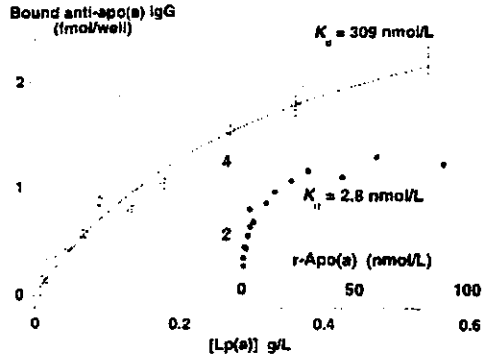

Figure 3. Isotherm of the binding to fibrin of r-apo(a) and Lp(a). Varying concentrations of purified r-apo(a) A30 and Lp(a) particles containing a similar-sized apo(a) isoform were incubated with the fibrin surface. After 3 washes, the fibrin surface was probed with a ^{125}I-radiolabeled polyclonal antibody directed against human apo(a). Radioactivity bound was then transformed into femtomoles of antibody bound to fibrin. The x-axis represents the concentrations of r-apo(a) (nmol/L) and Lp(a) (g/L) that were incubated with fibrin. The y-axis represents the mass of antibody bound, a quantity proportional to the amount of r-apo(a) A30 adsorbed onto fibrin, as described by Equation 2. Data were fitted to the Langmuir adsorption equation. The binding isotherm of Lp(a) is represented in the main figure, with K_d of 309 nmol/L, calculated by using molecular masses of 512 and 540 kDa for 30-kringle apo(a) and apoB-100, respectively. The binding of r-apo(a) A30 is shown in the inset (K_d 2.8 nmol/L). Bars represent the error of triplicates.

incubation of r-apo(a) and native apo(a) or Lp(a) with fibrin surfaces. Binding to these surfaces was quantified with a ^{125}I-labeled polyclonal IgG raised against r-apo(a) A10. The affinity of the purified IgG for the different r-apo(a)s was in the same order of magnitude (K_d 47±15 nmol/L) as measured by using surface plasmon resonance (data not shown). Therefore, the amount of ^{125}I-labeled IgG bound was assumed to be proportional to the amount of apo(a) bound according to Equation 2. All the isoforms tested (A10 to A34) showed dose dependence, saturation, and specificity for carboxy-terminal lysine residues of fibrin as indicated by its inhibition with 6-amino-hexanoic acid. Fitting of the data obeys the simple Langmuir equation (Equation 1) for adsorption at interfaces,[33] as indicated by typical binding isotherms for isolated r-apo(a) A30 and the corresponding Lp(a) reported in Figure 3. The calculated dissociation constant (K_d) values for the different r-apo(a)s were similar (3.6±0.3 nmol/L), regardless of their size (Table). In contrast, a clear inverse relationship between apo(a) size and affinity for fibrin could be documented for Lp(a) particles, in agreement with previous published data.[15,34] These results suggest that the LBS function of apo(a) is modified by the composite structure of the Lp(a) particle, as previously suggested by Klezovitch et al.[8] Indeed, the K_d (2.5 nmol/L) of a 15-kringle apo(a) separated from native Lp(a) was similar to the mean K_d (3.4 nmol/L) of the closest size r-apo(a) A14, thus indicating that the mild reductive conditions we used to separate apo(a) from native Lp(a) did not disturb its lysine-binding function. In contrast, the corresponding native Lp(a) particle displayed a 4-fold higher K_d (10.5 nmol/L), indicative of lower affinity. The highest differences in affinity were found between r-apo(a)

Affinity of r-apo(a) and Native Lp(a) for Fibrin

Isoforms	Affinity (K_d, nmol/L)*
r-apo(a)† A10 to A34	3.6±0.3†
Apo(a)‡ (15 K)	2.5±0.2‡
Lp(a)§ (15 K)	10.5±0.9§
Lp(a) (20 K)	44.6±4.4
Lp(a) (26 K)	165±5
Lp(a) (30 K)	309
Lp(a) (34 K)	541

*Dissociation constants (K_d) as calculated by fitting raw data to the Langmuir equation.

†Results are given as means±SD for 7 r-apo(a) isoforms differing by the number of K-IV type 2 from 0 (A10 kringles isoform) to 24 (A34 kringles isoform). At least three different experiments were performed in triplicate for each r-apo(a) isoform.

‡Apo(a) 15 kringles isolated from native Lp(a) by mild reduction according to Edelstein et al.[27]

§Values for Lp(a) particles as mean±SD when indicated. Other values represent the mean of experiments performed in triplicate. Lp(a) was purified from human plasma as indicated. The plasmas were obtained from volunteers attending the outpatient clinic of the Centre Hospitalo-Universitaire de Reims (France).

A30 (K_d 3.6 nmol/L) and r-apo(a) A34 (K_d 2.1 nmol/L) and their corresponding native Lp(a) particles (Table).

All r-apo(a)s inhibited the binding of plasminogen to fibrin with a similar intensity (Figure 4). The amount of plasminogen bound to fibrin decreased as a function of the input concentration of r-apo(a) that was added, and it attained 50% (IC_{50}) at 16.8±5.4 nmol/L These results are indicative of competitive inhibition between plasminogen and r-apo(a) for the same type of binding sites. At identical molar concentrations, the size of the different r-apo(a) preparations had no influence on the competitive binding.

Lp(a) and r-Apo(a) Fibrin-Binding Diversity Is Equally Neutralized by LBS-Blocking mAb

Binding of the different-sized r-apo(a) preparations and of native plasma Lp(a) particles was efficiently inhibited by a

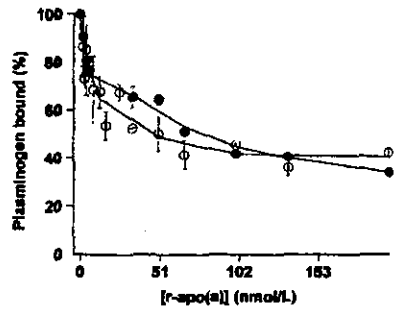

Figure 4. Inhibition of the binding of plasminogen to fibrin by r-apo(a) of different sizes. Varying amounts (0 to 200 nmol/L) of the different r-apo(a)s were incubated with a constant amount (~4 nmol/L) of ^{125}I-plasminogen onto the fibrin surface, and bound radioactivity was counted in a γ-counter. Bound plasminogen, represented as a percentage of the total amount bound in the absence of r-apo(a), is plotted against the concentration of r-apo(a) that was added. Bars represent the error of duplicates. To simplify the figure, only the effect of r-apo(a) A10 (○) and r-apo(a) A18 (●) is shown. The mean±SD IC_{50} value for all r-apo(a)s tested was 16.8±5.4 nmol/L.

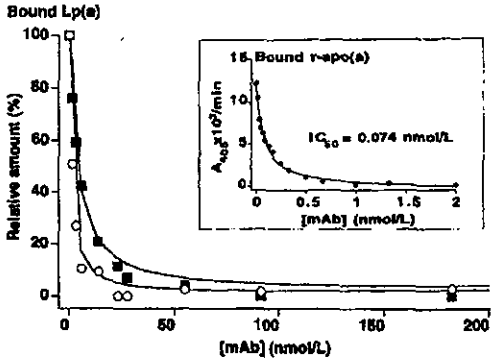

Figure 5. Inhibition of the binding of plasma Lp(a) and r-apo(a) A10 to fibrin by mAb A10.2. In the main graph, plasma samples from 2 subjects [1.2 g/L Lp(a), 10-kringle apo(a) isoform (○); 2.2 g/L Lp(a), 19- and 28-kringle apo(a) isoforms (■)] were incubated with varying concentrations of mAb A10.2 (0 to 545 nmol/L, by protein assay[25] and with M_r 150 000). The inset shows a fixed concentration (1 nmol/L) of r-apo(a) A10 (●) incubated with varying concentrations (0 to 2 nmol/L) of mAb A10.2. The solutions were then incubated with fibrin. Unbound proteins were discarded, and bound r-apo(a) or Lp(a) was detected with a polyclonal antibody directed against r-apo(a). The concentration of mAb that inhibits 50% (IC_{50}) of the binding of r-apo(a) (inset) and plasma Lp(a) (main graph) to fibrin was calculated from raw data by using a modified hyperbolic decay equation derived from the original Langmuir equation. The IC_{50} calculated for r-apo(a) was 0.074 nmol/L, whereas the corresponding value for plasma Lp(a) was 3.9 (○) and 4.2 nmol/L (■). An unrelated mAb (7D4) directed against plasminogen activator inhibitor (PAI)-1, which was used as control at identical concentrations, produced no modification on the binding of either r-apo(a) or Lp(a) to fibrin. To simplify the graphs, only the effect of mAb A10.2 is shown.

mAb directed against the LBS of K-IV type 10.[20] Inhibition of the binding was dose dependent (Figure 5), and an IC_{50} of 0.074 nmol/L was calculated for r-apo(a) from data displayed in the inset to Figure 5. The IC_{50} values for plasma, shown in Figure 5, were 3.9 nmol/L for the inhibition of a plasma Lp(a) containing a 10-kringle apo(a) isoform and 4.2 nmol/L for an Lp(a) containing 19- and 28-kringle apo(a) isoforms. These IC_{50} values were higher than those obtained with the isolated r-apo(a) and most probably reflect competition/consumption by kringle 4 of plasma plasminogen, which is also recognized by mAb A10.2.

Discussion

Definition of a threshold risk value for Lp(a) is complicated by variations in the distribution of Lp(a) among different populations.[36] However, most studies have accepted an Lp(a) concentration >30 mg/dL as a major independent risk factor for atherosclerosis.[1] The circulating concentration of Lp(a) is mainly regulated by the apo(a) gene, which varies in size as a function of the number of repetitive sequences encoding K-IV type 2. The smaller this hypervariable region, the higher the plasma concentration of Lp(a) will be. Thus, short apo(a) alleles may favor atherogenesis by increasing the concentration of Lp(a). In agreement with this known inverse relationship between the size of apo(a) isoforms and plasma Lp(a) concentration, a series of studies have shown a prevalence of small-sized apo(a) isoforms in coronary heart disease patients[17,18,37-40] or in those with advanced stenotic atherosclerosis.[19] However, it remains to be determined whether short apo(a) alleles are, per se, an atherosclerotic risk factor.[41] The possibility that apo(a) phenotypes may have different functional properties regarding fibrin and cell binding has been explored; an apo(a) size–related variability in affinity has previously been reported with the use of native Lp(a) particles.[14,16,34] Because the main structural difference between apo(a) isoforms almost certainly reflects a variable number of K-IV type 2 repeats, the present study was aimed at determining their effect on fibrin binding. We sought to explore this phenomenon by comparing binding to fibrin of different-sized r-apo(a) and Lp(a) particles. The r-apo(a)s differed solely by the number of copies of K-IV type 2 and were similar regarding lysine-binding kringle structures. Binding of the purified r-apo(a) and Lp(a) particles to fibrin inhibited plasminogen binding and was specific for lysine residues. Of note, LDL particles were shown to be unable to modify plasminogen binding to fibrin. Furthermore, binding to fibrin of r-apo(a) and Lp(a) in plasma was neutralized with a mAb that specifically recognizes the fibrin-binding function of apo(a) K-IV type 10.[20] It has been reported that regions constituting K-IV type 5 to K-IV type 8[8] and K-V-protease[42] may also be involved in fibrin binding. These regions are present in all the r-apo(a) that we have tested, and it is possible that mAb A10.2 may also neutralize the corresponding LBS.

The dissociation constant of the different r-apo(a) for fibrin was in the same order of magnitude (3.6±0.3 nmol/L) as the value previously determined with a radiolabeled r-apo(a) containing 17 K-IV copies.[35] These values are ≈150- to 250-fold lower than the K_d value that we have determined for plasminogen in previous reports (0.5 to 1 μmol/L)[16,29] and in the present study (0.9 μmol/L). These differences in K_d values explain the ability of r-apo(a) to compete efficiently with plasminogen for binding to fibrin. Most interesting was the finding of quantitative differences in affinity between the r-apo(a) and Lp(a) preparations tested. The functional heterogeneity of native Lp(a) particles is in contrast to the homogeneity in K_d values found for all the r-apo(a)s tested and for the isolated native 15-kringle apo(a). Thus, the purified Lp(a) particles displayed lower affinities for fibrin than did the corresponding r-apo(a)s. Of greater interest was the observation that the change in affinity for the 15-kringle apo(a) isolated from native Lp(a) was moderate (4-fold) compared with the important difference in affinity (85-fold) observed between the r-apo(a) A30 and the corresponding Lp(a) particle. The study of other apo(a) phenotypes derived from reduced Lp(a) particles is hampered by the risk of severely affecting the LBS structure and function by sulfhydryl groups.[43]

Our data suggest that the fibrin affinity of small-sized apo(a) isoforms is preserved in Lp(a), whereas the affinity for fibrin of high-molecular-mass isoforms (>22 kringles) is reduced in a size-dependent manner in the Lp(a) particle. We hypothesize, in agreement with Klezovitch et al,[8] that interactions of apo(a) kringles with components of the Lp(a) particle may contribute to the observed changes in fibrin affinity. Indeed, Ernst et al[9] have shown that binding to lysine-Sepharose of apo(a) incorporated into an LDL particle

was strongly decreased. These modifications in affinity may be related to conformational changes. As a matter of fact, the extended shape of free r-apo(a)[44] in solution is in contrast to the natural roughly spherical shape of Lp(a), where apo(a) is closely associated with the surface, as recently visualized by Weisel et al.[45] On the other hand, homocysteine has been shown to induce a concentration-dependent increase in its affinity for fibrin[46] and a concomitant release of free apo(a) from reduced Lp(a). According to our present results, the free apo(a) released by homocysteine may contribute to the observed increase in the fibrin affinity of Lp(a) through the "multiple binding with identical linkage mechanism."[34]

Taken altogether, the results presented in the present study demonstrate that in contrast to native Lp(a) particles, all r-apo(a)s tested display a size-independent similar affinity and specificity for lysine residues on fibrin. Therefore, affinity for fibrin appears to be an intrinsic property of apo(a) that is not dependent on kringle number, except for apo(a) isoforms constitutive of an Lp(a) particle.

Acknowledgments

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Mutations and common polymorphisms in *ADAMTS13* gene responsible for von Willebrand factor-cleaving protease activity

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von Willebrand factor (VWF) is synthesized primarily in vascular endothelial cells and secreted into the plasma as unusually large VWF multimers. Normally, these multimers are quickly degraded into smaller forms by a plasma metalloproteinase, VWF-cleaving protease (VWF-CP). Decreases in the activity of this enzyme result in congenital and acquired thrombotic thrombocytopenic purpura (TTP). The human VWF-CP has recently been purified. Cloning of the corresponding cDNA revealed that the 1,427-aa polypeptide is a member of the ADAMTS gene family, termed *ADAMTS13*. Twelve rare mutations in this gene have been identified in patients with congenital TTP. Here, we report missense and nonsense mutations in two Japanese families with Upshaw–Schulman syndrome, congenital TTP with neonatal onset and frequent relapses. The comparison of individual *ADAMTS13* genotypes and plasma VWF-CP activities indicated that the R268P, Q449stop, and C508Y mutations abrogated activity of the enzyme, whereas the P475S mutant retained low but significant activity. The effects of these mutations were further confirmed by expression analysis in HeLa cells. Recombinant VWF-CP containing either the R268P or C508Y mutations was not secreted from cells. In contrast, Q449stop and P475S mutants were normally secreted but demonstrated minimal activity. Genotype analysis of 364 Japanese subjects revealed that P475S is heterozygous in 9.6% of individuals, suggesting that approximately 10% of the Japanese population possesses reduced VWF-CP activity. We report on a single-nucleotide polymorphism associated with alterations in VWF-CP activity; it will be important to assess this single-nucleotide polymorphism as a risk factor for thrombotic disorders.

Thrombotic thrombocytopenic purpura (TTP) is a generalized disorder characterized by microangiopathic hemolytic anemia, thrombocytopenia, neurological dysfunction, renal failure, and fever (1–3). Mortality of patients afflicted with this condition may exceed 90% without plasma exchange, the only effective treatment of TTP currently. Both acquired and congenital types of TTP exist. About half of patients with congenital TTP have their first acute episode in childhood, whereas the other half have their first acute episode in adulthood. Symptoms in adults often develop in association with the stress of infection or pregnancy (4). TTP with neonatal onset and frequent relapses is often diagnosed as Upshaw–Schulman syndrome (USS) (5, 6).

The platelet-adhesive blood coagulation protein, von Willebrand factor (VWF), is synthesized mainly in vascular endothelial cells (7–10). VWF is released into plasma as "unusually large" multimeric forms (UL-VWFM), highly active in interactions with platelets and collagen (11, 12). In normal human plasma, UL-VWFM rapidly depolymerizes to smaller multimeric forms ranging in size from 500 to 20,000 kDa. In patients with TTP, UL-VWFM remains in the circulation, because of the loss of regulated VWF proteolysis. The binding of UL-VWFM to platelets may then promote microvascular thrombosis, platelet consumption, and hemolysis. Enhanced platelet aggregation under high shear stress was also proposed as the mechanism of the clinical symptoms of TTP (13). Proteolytic depolymerization of VWF is catalyzed by VWF-cleaving protease (VWF-CP), partially purified by Furlan *et al.* (14) and Tsai (15). The cleavage site in VWF is an Y842-M843 peptidyl bond within the central A2 domain (14–17).

Human VWF-CP has recently been purified, allowing the determination of an N-terminal amino acid sequence (18–20). This information identified the cDNA sequence encoding VWF-CP (18, 21). Additional approaches attempting to determine the genetic cause of congenital TTP also discovered the same gene, *ADAMTS13* (22). VWF-CP/ADAMTS-13 is a member of the ADAMTS family of metalloproteases, named for the characteristic combination of a disintegrin-like and metalloprotease with thrombospondin type 1 (TSP1) motif (23–26). VWF-CP is predominantly expressed in liver (18, 21, 22, 25), consistent with our previous observation that severely decreased levels of plasma VWF-CP activity in patients with biliary atresia can be restored by living-related liver transplantation.‡‡ VWF-CP is 1,427 aa residues in length, containing a signal peptide, a propeptide, a reprolysin-like metalloprotease domain, a disintegrin-like domain, a TSP1, a cysteine-rich domain, seven additional TSP1 repeats, and two CUB domains identified in many developmentally regulated proteins (27). The C terminus of the VWF-CP propeptide ends with the sequence RQRR, suggesting that furin or a related protease cleaves this region during synthesis to allow the secretion of VWF-CP into the plasma as an active enzyme.

The human *ADAMTS13* gene contains 29 exons, spanning ≈37 kb on chromosome 9q34 (18, 21, 22). Levy *et al.* (22) identified 12 mutations of *ADAMTS13* in seven families of patients affected by congenital TTP (22). Two frameshift mutations were identified in exons 19 and 27, and one splice mutation was found in intron 13. The remaining nine mutations all result in nonconservative amino acid substitutions (H96D, R102C, T196I, R398H, R528G, R692C, C951G, C1024G, and C1213Y). These 12 mutations were excluded as common sequence polymorphisms. They also identified seven single-nucleotide polymorphisms (SNPs) associated with amino acid substitutions (R7W, Q448E, P618A, R625H, A732V, A900V,

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Abbreviations: TTP, thrombotic thrombocytopenic purpura; USS, Upshaw–Schulman syndrome; VWF, von Willebrand factor; UL-VWFM, unusually large VWF multimer; VWF-CP, VWF-cleaving protease; TSP1, thrombospondin-1; SNP, single-nucleotide polymorphism; PNGase F, peptide:N-glycosidase F; WT, wild type.

See commentary on page 11552.

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