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Original Article

Antiplatelet Loading Effects Prior to Endovascular Treatment: Carotid Artery Stenosis versus Unruptured Cerebral Aneurysms

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Objective: Carotid artery stenosis and cerebral aneurism may have different platelet functions and antiplatelet responses because these diseases have different etiologies. In this study, we compared the antiplatelet loading effects prior to endovascular treatment between carotid artery stenosis and unruptured cerebral aneurysm (UCA) patients.
Methods: Nine patients with asymptomatic carotid artery stenosis (ACS), 14 with symptomatic carotid artery stenosis (SCS), and 20 with unruptured cerebral aneurysms were enrolled in this study. Antiplatelet (aspirin + clopidogrel) loading effects prior to endovascular treatment were evaluated using light transmission aggregometry and platelet aggregate detection methods.
Results: Although there are differences in the prevalence of atherosclerosis risk factors, maximum aggregation rates in light transmission aggregometry and platelet aggregation-prone properties were not different in the three disease groups.
Conclusion: Preoperative dual antiplatelet therapy with aspirin and clopidogrel may be appropriate for both carotid artery stenosis and cerebral aneurism patients even though their conditions and background factors differ.

Keywords ▶ carotid artery stenosis, unruptured cerebral aneurysm, platelet aggregation, light transmission aggregometry, platelet aggregate

Introduction

In patients with carotid artery stenosis, the possibility that the effects of antiplatelets administered to prevent thrombotic complications of endovascular treatment are reduced compared with patients with unruptured cerebral aneurysms (UCA), which are not an atherosclerotic disorder, cannot be excluded because of the possible intrinsic increase in platelet function due to atherosclerosis,[1)] and the high prevalence of diabetes mellitus[2)] and dyslipidemia,[3)] which may attenuate the effects of aspirin. However, whether standard preoperative dual antiplatelet treatment with aspirin and clopidogrel is similarly effective in these diseases with different pathological features and background factors has not been evaluated. In this study, we investigated whether preoperative dual antiplatelet therapy with aspirin and clopidogrel causes differences in antiplatelet effects during endovascular treatment between patients with carotid artery stenosis and those with UCA, who are likely to have intrinsic differences in platelet function and antiplatelet responses.

Materials and Methods

The subjects were 43 patients who received elective endovascular treatment and preoperative dual antiplatelet therapy with aspirin and clopidogrel at our hospital between December 2016 and June 2018, and in whom platelet function was able to be assessed. The patients were registered prospectively. They consisted of 9 with asymptomatic carotid artery stenosis (ACS group), 14 with symptomatic carotid artery stenosis (SCS group), and 20 with UCA group. During the same period, a total of 9 patients with ACA, 15 with SCS, and 21 with UCA were treated at our

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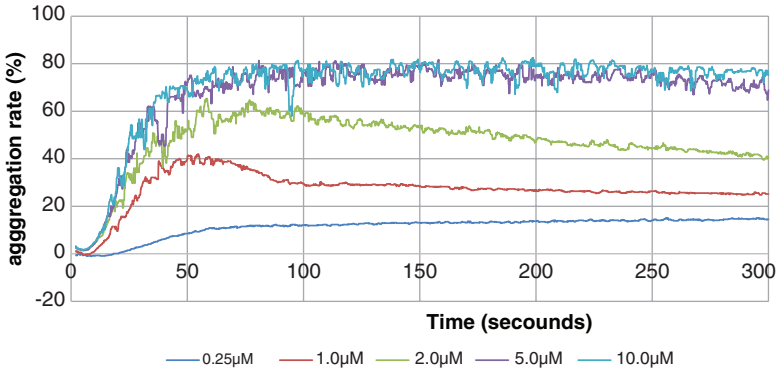

Fig. 1 Aggregation curves by light transmission aggregometry at different ADP concentrations. The horizontal axis represents time (sec) and the vertical axis represents the aggregation rate (%). The reaction time until the maximum aggregation rate was set at 300 seconds.[4,8] Although not shown in the graph, similar curves were drawn by collagen-induced and arachidonic acid-induced aggregation. The reaction time to maximum aggregation was 300 seconds in collagen-induced aggregation and 270 seconds in arachidonic acid-induced aggregation.[4,8]

hospital. Platelet function was evaluated by administering 100 mg of aspirin and 75 mg of clopidogrel for 7–10 days (ACS and UCA groups) or for 14–21 days from the period of acute-phase treatment (SCS group) prior to treatment. In this study, to minimize errors associated with measurement conditions, platelet function was measured in the three groups by restricting the period and by complete standardization of not only the measurement conditions but also the measurer. Treatment was performed on the day of or the day after the evaluation of platelet function.

In this study, platelet aggregates formed on a full-automatic hematology analyzer were simultaneously evaluated in addition to standard light transmission aggregometry to assess platelet function from multiple viewpoints.[4] Platelet aggregability was examined by the light transmission method using CS2000i (Sysmex Corporation, Kobe, Japan). Blood was collected from the median cubital vein using a plastic syringe (Terumo, Tokyo, Japan) and a 21-G needle (Terumo) on the day before or in the morning on the day of endovascular treatment. Platelet-rich plasma (PRP) to be analyzed by light transmission aggregometry was prepared by centrifuging blood collected in a citrate tube at 85 × g for 15 minutes. To eliminate the ceiling or floor effects, platelet aggregation inducers were used at the following concentrations: AD P at 0.25 μM, 1.0 μM, 2.0 μM, 5.0 μM, and 10 μM; collagen at 0.05 μg/mL, 0.5 μg/mL, 2.0 μg/mL, 5.0 μg/mL, and 1.0 μg/mL; arachidonic acid at 0.1 mM, 1.0 mM, and 1.2 mM (**Fig. 1**). Comparisons among the three groups were made at 5 μM[5] and 10 μM[6] ADP, 2.0 μg/mL collagen, and 1.2 mM arachidonic acid, that is, the concentrations at which no ceiling or floor effects on the analysis

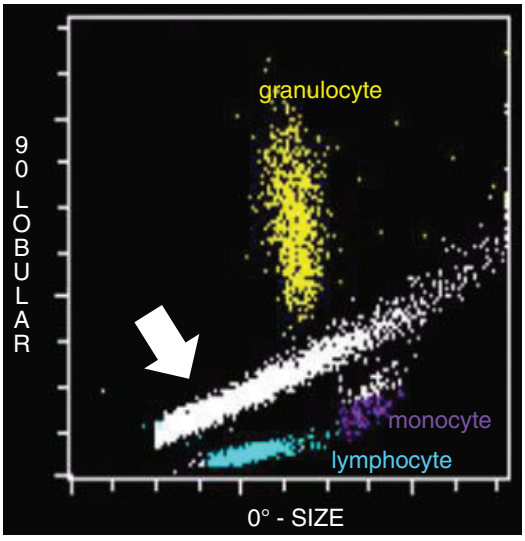

Fig. 2 Detection of platelet aggregates using a fully automatic hematology analyzer. The horizontal axis represents the size of blood cells and aggregates, and the vertical axis represents the degree of nuclear lobulation and complexity of aggregates. The white arrow indicates the territory of platelet aggregates.

were observed (**Fig. 1**). Platelet aggregates were detected using a full-automatic hematology analyzer (CELL-DYN Sapphire Hematology System; Abbott Diagnosis, Abbott Park, IL, USA). Platelet aggregates by blood collection maneuver were excluded using blood collected simultaneously in an ethylenediaminetetraacetate (EDTA) tube. In this study, according to the previous reports,[7,8] the analytical procedure was repeated a maximum of four times until platelet aggregates (**Fig. 2**) formed, and the number of assays until aggregates were first detected was compared. By this analytical method, platelet aggregates are detected in an earlier assay (1st or 2nd) if platelet function is

Table 1 Comparison of the patient background

	ACS (9 patients)	SCS (14 patients)	UCA (20 patients)	P value	ACS vs SCS	ACS vs UCA	SCS vs UCA
Age (years)	72.9 ± 4.5	72.4 ± 6.8	59.0 ± 12.3	<0.01	0.99	<0.01	<0.01
Sex: M:F	8:1	16:0	4:16	<0.01	0.62	<0.01	<0.01
Hypertension	8 (89%)	12 (75%)	10 (50%)	0.11			
Diabetes	4 (45%)	9 (56%)	1 (5%)	<0.01	0.69	<0.01	<0.01
Dyslipidemia	4 (45%)	14 (88%)	8 (40%)	<0.01	0.06	1	<0.01
Drinking	5 (56%)	5 (31%)	4 (20%)	0.16			
Smoking	5 (56%)	6 (38%)	1 (5%)	<0.01	0.43	<0.01	0.03

ACS: asymptomatic carotid artery stenosis; SCS: symptomatic carotid artery stenosis; UCA: unruptured cerebral aneurysm

Table 2 Comparison of the maximum aggregation rate by light transmission aggregometry

	ACS	SCS	UCA	P value
ADP (5 μM)	69.8 ± 9.0	67.1 ± 18.0	66.7 ± 13.0	0.739
ADP (10 μM)	69.2 ± 9.2	70.9 ± 8.0	65.1 ± 12.2	0.641
Collagen	56.8 ± 27.2	63.2 ± 18.0	52.3 ± 24.7	0.566
Arachidonic acid	11.7 ± 7.7	13.4 ± 8.3	12.5 ± 17.7	0.362

ACS: asymptomatic carotid artery stenosis; ADP: adenosine diphosphate; SCS: symptomatic carotid artery stenosis; UCA: unruptured cerebral aneurysm

increased but in a later assay (3rd or 4th), or not detected even in the 4th assay, if it is decreased.[7,8]

Statistically, the background of the subjects was compared by the Kruskal–Wallis or Fisher's exact test, and concerning the items that significantly differed, the differences among them were examined by the Steel–Dwass test for multiple comparisons. The results of light transmission aggregometry were compared among the three groups using the Kruskal–Wallis test, and the results of platelet aggregate detection were compared using the chi-square test. The statistical software EZR (Jichi Medical University Saitama Medical Center, Saitama, Japan)[9] was used.

This study was approved by the institutional review board of our hospital (approval No. 141501201). Written informed consent was received from all patients.

Results

Regarding the patient background, the age (P <0.01), the percentage of males (P <0.01), and the percentage of those with risk factors of atherosclerosis (diabetes, dyslipidemia, and smoking) were significantly higher (P <0.01) in the SCS and ACS groups than in the UCA group (**Table 1**). However, in the ACS, SCS, and UCA groups, the maximum platelet aggregation rate determined by the light transmission method was 69.8%, 67.1%, and 66.7% with adenosine diphosphate (ADP) (5 μM), 69.2%, 70.9%, and 65.1% with ADP (10 μM), 56.8%, 63.2%, and 52.3% with collagen (2.0 μg/mL), and 11.7%, 13.4%, and 12.5% with arachidonic acid (1.0 mM), respectively, demonstrating no significant difference among the three groups with any of the aggregation inducers (**Table 2**) (P >0.05). Moreover, the number of assays until platelet aggregates were first detected on the hematology analyzer (**Table 3**) did not differ among the three groups (P = 1.00).

Discussion

In this study, platelet function on the day before or the day of endovascular treatment, whether it was measured by the light transmission aggregometer or hematology analyzer, was similar between patients with carotid artery stenosis and those with UCA.

Thrombotic complications are the most frequent complications of neuroendovascular treatment, and they develop in about 2% of the patients undergoing carotid artery stenting for symptomatic and ACS[10–12] and in 4%–8.2% of the patients undergoing coil embolization for UCA.[13–15] Reducing thrombotic complications is important for improving the results of neuroendovascular treatment. Presently, dual antiplatelet therapy with aspirin and clopidogrel is standard preoperative management for endovascular treatment of carotid artery stenosis and cerebral aneurysms as an empirical treatment.[16] However, whether uniform dual antiplatelet therapy is equally effective in these primary diseases differing in pathology and background factors has not been evaluated.

Table 3 Comparison of the number of assays until the first detection of platelet aggregates using a fully automatic hematology analyzer

Number of assays	ACS	SCS	UCA
1st, 2nd	1 (11%)	2 (14%)	3 (15%)
3rd, 4th	4 (45%)	6 (43%)	9 (45%)
Not detected even on the 4th assay	4 (45%)	6 (43%)	8 (40%)
Total	9 (100%)	14 (100%)	20 (100%)

ACS: asymptomatic carotid artery stenosis; SCS: symptomatic carotid artery stenosis; UCA: unruptured cerebral aneurysm

Diabetes mellitus[2] and hypercholesterolemia,[3] which are risk factors for atherosclerosis, are factors of aspirin resistance, and smoking increases the antiplatelet effects of clopidogrel.[17] Therefore, in carotid artery stenosis, which is an atherosclerotic disorder, they may affect the effects of antiplatelets. However, as cerebral aneurysms, which are a non-atherosclerotic disorder, are unlikely to be affected by these factors, the effects of antiplatelets may differ from those in carotid artery stenosis. If the effect of uniformly administrated antiplatelet varies according to the disease condition and background factors, the method of antiplatelet administration for the prevention of thrombotic complications in endovascular treatment must be changed according to the disease condition and background factors. On the other hand, if a similar degree of platelet suppression with sufficient preventive effects can be obtained by uniform antiplatelet administration regardless of the disease state or background, it is information of high clinical value because routine measurement of platelet function is impossible at many facilities.

Although the gold standard of platelet function testing is light transmission aggregometry, increased platelet aggregability cannot be detected by the light transmission method alone.[7] In this study, therefore, we simultaneously examined platelet aggregates using a hematology analyzer.[4] Following previous reports, platelet aggregates on scattergrams formed by automatic stirring in a full-automatic hematology analyzer were examined.[7,8] The maximum aggregation rate determined by the light transmission method under the same conditions at our facility in 65 healthy individuals (mean age: 44.7 years) was 89.7% with ADP (5 μM), 87.3% with ADP (10 μM), 91.7% with collagen (2.0 μg/mL), and 91.1% with arachidonic acid (1.0 mM),[7] and the frequency of detection of platelet aggregates with the hematology analyzer on the 1st or 2nd assay was 29.4% (in this study, 11% in the ACS group, 14% in the SCS group, and 15% in the UCA group).[7] Therefore, it was confirmed that the antiplatelets sufficiently suppressed platelet function and that the evaluation items of platelet function selected in this study were not subject to the ceiling or floor effects. Thus, this study suggested that generally consistent antiplatelet effects can be obtained in both patients with carotid artery stenosis and in those with UCA by dual antiplatelet therapy for a given period regardless of differences in the disease state and background factors.

In patients with carotid artery stenosis or UCA, there have been reports of evaluation of the effects of antiplatelets administered for endovascular treatment in each disease[18,19] and reports of evaluation using a single testing method,[20] but there has been no evaluation of platelet function before treatment in these diseases from multiple viewpoints before this study. It is impossible to directly compare the results obtained in separate studies in patients with carotid artery stenosis[18] and in patients with UCA.[19] Furthermore, the results of evaluation by a single testing method may be markedly affected by specific background factors depending on the characteristics of the test.[20] In contrast, more clinically faithful and accurate results are obtained by directly comparing platelet function in patients with carotid artery stenosis with that in those with UCA from multiple viewpoints, as in this study. Therefore, based on this study, the effects of dual antiplatelet therapy before endovascular treatment are similar between carotid artery stenosis and UCA patients, and alteration of the strength of preoperative antiplatelet therapy before endovascular treatment for these disorders in consideration of background factors may be unnecessary.

Although this study has advantages such as that it was a prospective observational study in which nearly all patients treated during the observation period (95.6%) were registered and that platelet function was comparatively evaluated from multiple viewpoints, the small number of registered patients was a limitation. Another limitation is that differences in the effects of antiplatelets according to the disease state and background factors in each patient were not evaluated. However, this study supports prophylactic administration of two antiplatelets in combination before endovascular treatment, which has been practiced empirically for both carotid artery stenosis and UCA, and suggested its clinical usefulness in consideration of the

present state that routine platelet function testing is difficult at many facilities. To confirm the results of this study, evaluation in a larger number of patients, including gene polymorphism of each patient, is necessary. In addition, if rapid assessment of background factors of each patient, including gene polymorphism, becomes possible at all facilities in the future, adjustment of prophylactic antiplatelet administration according to the disease state and background factors may become necessary.

Conclusion

In endovascular treatment of carotid artery stenosis and asymptomatic cerebral aneurysms, nearly comparable platelet suppression can be achieved by the dual antiplatelet therapy with aspirin and clopidogrel, which has been practiced empirically, regardless of the differences in the disease state and background factors.

Disclosure Statement

The authors declare no conflict of interest.

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