the two groups. The number of our patients might be too small to find differences between the two groups.

Systolic and diastolic blood pressures on admission were higher in Group D than Group N. Dávalos et al. [17] reported that high systolic blood pressure on admission was independently related with early deterioration after ischemic stroke. Whereas, Jørgensen et al. [18] showed that high systolic blood pressure on admission decreased risk for early progression. In the present study, the exact relationship between high blood pressure and neurological deterioration is unknown. A further study will be needed to solve the issue.

In the present study, NIHSS score and the body temperature was higher in Group D than in Group N, but these difference was not significant. Patients with high NIHSS score at admission were likely to have neurological deterioration in acute phase of ischemic stroke [2,19]. A few investigators reported that patients with higher temperature had a worse stroke outcome [20–22]. However, it has still unknown whether higher temperature is associated with early deterioration at acute phase of ischemic stroke.

In our study, the follow-up DWI study in all the patients but one with neurological deterioration revealed new small infarcts addition to the initial infarcts. Therefore, we concluded that recurrence of small infarcts resulted in neurological deterioration. In patients with small non-lacunar infarcts, prevention of recurrent infarcts is important for avoiding neurological deterioration.

A number of problems were present in this study. Firstly, there was a small sample size of deterioration patients with a follow-up DWI study. Therefore, statistic analysis was weak. Secondly, we could not conduct follow-up DWI studies in many patients without neurological deterioration. Therefore, we could not exclude the possibility that new but asymptomatic lesions appeared if follow-up DWI in those patients is performed.

In conclusion, our study demonstrated that the frequency of neurological deterioration in patients with small non-lacunar infarcts was 13% within 7 days after symptom onset. Neurological deterioration in these patients was frequently accompanied by recurrent infarction visualized with DWI.

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References

[1] Dávalos A, Castillo J. Progressing stroke. In: Fisher M, Bogousslavsky J, editors. Current Review of Cerebrovascular Disease. Philadelphia (PA): Current Medicine Inc; 1999. p. 149–60.

[2] Dávalos A, Toni D, Iweins F, Lesaffre E, Bastianello S, Castillo J, et-al., for the ECASS Group. Neurological deterioration in acute ischemic stroke. Potential predictors and associated factors in European cooperative acute stroke study (ECASS) I. Stroke 1999;30: 2631–6.

[3] Serena J, Leira R, Castillo J, Pumar JM, Castellanos M, Dávalos A. Neurological deterioration in acute lacunar infarctions: the role of excitatory and inhibitory neurotransmitters. Stroke 2001;32: 1154–61.

[4] Nakamura K, Saku Y, Ibayashi S, Fujishima M. Progressive motor deficits in lacunar infarction. Neurology 1999;52:29–33.

[5] Lodder J, Gorsselink EL. Progressive stroke caused by CT-verified small deep infarcts; relation with the size of the infarct and clinical outcome. Acta Neurol Scand 1985;71:328–30.

[6] Yonemura K, Kimura K, Minematsu K, Uchino M, Yamaguchi T. Small centrum ovale infarcts on diffusion-weighted magnetic resonance imaging. Stroke 2002;33:1541–4.

[7] Wada K, Kimura K, Minematsu K, Uchino M, Yamaguchi T. Spotty cortical enhancement detected by magnetic resonance imaging: a sign of embolic transient ischemic attack and stroke? J Stroke Cerebrovasc Dis 2001;10:19–22.

[8] Warach S, Gaa J, Siewert B, Wielopolski P, Edelman RR. Acute human stroke studied by whole brain echo planar diffusion-weighted magnetic resonance imaging. Ann Neurol 1995;37:231–41.

[9] Tatu L, Moulin T, Bogousslavsky J, Duvernoy H. Arterial territories of human brain. Cerebral hemispheres. Neurology 1998;50: 1699–708.

[10] Bogousslavsky J, Regli F. Centrum ovale infarcts: subcortical infarction in the superficial territory of the middle cerebral artery. Neurology 1992;42:1992–8.

[11] Brott T, Adams Jr HP, Olinger CP, Marler JR, Barsan WG, Biller J, et al. Measurements of acute cerebral infarction: a clinical examination scale. Stroke 1989;20:864–70.

[12] Swieten JC, Koudstaal PJ, Visser MC, Schouten HJA, Gijn J. Interobserver agreement for the assessment of handicap in stroke patients. Stroke 1988;19:604–7.

[13] North American Symptomatic Carotid Endarterectomy Trial Collaborators. Beneficial effect of carotid endarterectomy in symptomatic patients with high-grade carotid stenosis. N Engl J Med 1991;325: 445–53.

[14] Koga M, Kimura K, Minematsu K, Yamaguchi T. Diagnosis of internal carotid artery stenosis greater than 70% with power doppler duplex sonography. AJNR Am J Neuroradiol 2001;22:413–7.

[15] Amarenco P, Cohen A, Tzourio C, Bertrand B, Hommel M, Besson G, et al. Atherosclerotic disease of the aortic arch and the risk of ischemic stroke. N Engl J Med 1994;331:1474–9.

[16] Toni D, Fiorelli M, Gentile M, Bastianello S, Sacchetti ML, Argentito C, et al. Progressing neurological deficit secondary to acute ischemic stroke. A study on predictability, pathogenesis, and prognosis. Arch Neurol 1995;52:670–5.

[17] Dávalos A, Cendra E, Teruel J, Martinez M, Genis D. Deteriorating ischemic stroke: risk factors and prognosis. Neurology 1990;40: 1865–9.

[18] Jørgensen HS, Nakayama H, Raaschou HO, Olsen TS. Effect of blood pressure and diabetes on stroke in progression. Lancet 1994;344: 156–9.

[19] DeGraba TJ, Hallenbeck JM, Pettigrew KD, Dutka AJ, Kelly BJ. Progression in acute stroke. Value of the initial NIH Stroke Scale Score on patient stratification in future trials. Stroke 1999;30:1208–12.

[20] Azzimondi G, Bassein L, Nonino F, Fiorani L, Vignatelli L, Re G, et al. Fever in acute stroke worsens prognosis: a prospective study. Stroke 1995;26:2040–3.

[21] Castillo J, Dávalos A, Marrugat J, Noya M. Timing for fever-related brain damage in acute ischemic stroke. Stroke 1998;29:2455–60.

[22] Wang Y, Lim LL, Levi C, Heller RF, Fisher J. Influence of admission body temperature on stroke mortality. Stroke 2000;31:404–9.

Vertebral Artery Occlusion in Duplex Color-Coded Ultrasonography

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Background and Purpose—To establish the diagnostic criteria for the site of occlusion in the vertebral arteries (VAs) using duplex color-coded ultrasonography.

Methods—In 128 consecutive patients who underwent conventional cerebral angiography, we prospectively measured the diameter, mean flow velocity (MV), peak systolic flow velocity, and end-diastolic flow velocity of both VAs. The diameter-ratio (diameter of contralateral VA divided by that of target VA) and MV-ratio (MV of contralateral VA divided by that of target VA) were determined. Based on the angiographic findings, we classified the VAs into 4 types (5 groups) as follows: (1) the origin of VA occlusion (Origin group: n=9); (2) VA occlusion before branching into the posterior inferior cerebellar artery (PICA) (Before group: n=10); (3A) symptomatic VA occlusion after branching into the PICA (After group: n=12); (3B) asymptomatic or hypoplastic occlusive VA after branching into the PICA (PICA end group: n=15); and (4) no significant occlusive lesions in the VA (Control group: n=194).

Results—No flow signals in the VAs apparently indicated the Origin group. Preserved peak systolic flow velocity but end-diastolic flow velocity of zero cm/s indicated the Before group. MV <18 cm/s and MV-ratio ≥1.4 indicated the PICA end group or After group. Furthermore, these groups could be distinguished as follows: a diameter-ratio <1.4 indicated the After group. A diameter-ratio ≥1.4 indicated the PICA end group. Either MV ≥18 cm/s or MV <18 cm/s in combination with MV-ratio <1.4 indicated the Control group.

Conclusion—Duplex color-coded ultrasonography can accurately diagnose the site of VA occlusion. (***Stroke.* 2004;35: 1068-1072.)**

Key Words: vertebral artery ■ occlusion ■ ultrasonography ■ ultrasonography, Doppler, duplex ■ diagnosis ■ vertebrobasilar circulation

Duplex color-coded ultrasonography is useful in the evaluation of occlusive lesions in the carotid[1-6] and vertebral[7-13] arteries (VAs) in acute stroke patients. The diagnostic criteria for occlusive lesions in the carotid arteries have been already established.[1,5] Duplex color-coded ultrasonography is also valuable to evaluate pathological VAs, such as VA occlusion,[13,14] subclavian steal phenomenon,[12,15-17] and vertebral arterial dissection.[18-21] The site of VA occlusions is divided into 3 groups: VA origin occlusions, VA occlusions before branching into the posterior inferior cerebellar artery (PICA), and VA occlusions after branching into the PICA. However, the diagnostic criteria in duplex ultrasonography for the site of VA occlusion remain unclear. Furthermore, a few VAs show asymptomatic occlusion or naturally hypoplastic VA ending at the PICA (PICA end).[22] The aim of the present study was to establish the criteria for determining the site of occlusion of VAs, including VAs ending at the PICA, using duplex color-coded ultrasonography.

Methods

We prospectively assessed the 256 VAs of 128 consecutive patients (91 men and 37 women, mean±SD; 63.4±12.2 years) admitted to the National Cardiovascular Center and who underwent intraarterial digital subtraction angiography (IA-DSA) between May 1, 2003 and July 31, 2003. We excluded 16 VAs with 50% to 99% stenosis in diameter on angiography because the flow velocity was also affected by the stenotic lesions. Therefore, 240 VAs were examined in the present study. Eighty-four patients had acute cerebral infarctions (33 in the vertebrobasilar circulation and 51 in the internal carotid arterial circulation), 12 had transient ischemic attacks, 20 had old infarctions (12 in the vertebrobasilar circulation, 8 in the internal carotid arterial circulation), 3 had cerebral hemorrhages, and the remaining 9 nonstroke patients had asymptomatic arterial stenotic or occlusive lesions (1 in the basilar artery, 2 in the middle cerebral artery, and 6 in the internal carotid artery). Eighty-four patients with acute stroke underwent IA-DSA within 2.6±3.9 days of stroke onset. Informed consent for IA-DSA was obtained from both the patient and family.

Selective IA-DSA was performed using a biplane, high-resolution angiography system (Angio Rex Super-G and DFP-2000A; Toshiba) with a matrix of 1024×1024 pixels. A catheter was inserted into the right brachial artery or femoral artery in accordance with the

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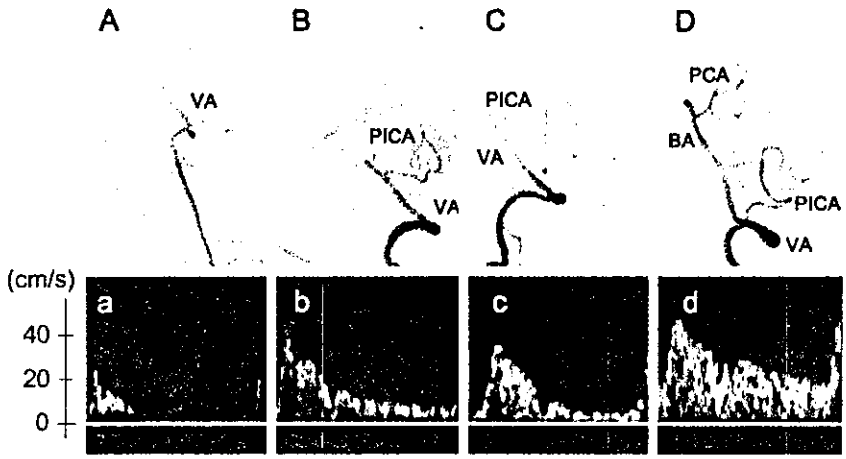

Figure 1. Angiogram (lateral view of vertebral arterial angiography) and Doppler waveforms of patients in the Before group (A and a), After group (B and b), PICA end group (C and c), and Control group (D and d). A and a, The VA was occluded before branching into the PICA. The Doppler waveform showed no EDV. B and b, The VA was occluded after branching into the PICA. The Doppler waveform showed EDV and MV was lower than those of the Control group. C and c, The VA ended in the PICA and did not continue to the union of the BA. The Doppler waveform also showed EDV and MV were lower than those of the Control group. D and d, No significant occlusion of the VA. The Doppler waveform showed EDV and MV was highest among all groups. VA indicates vertebral artery; BA, basilar artery; PICA, posterior inferior cerebellar artery; PCA, posterior cerebral artery.

Seldinger method, and then guided to the cerebral arteries for diagnostic 4-vessel angiography. Based on the angiographic findings, we classified the VA vessels into 4 types (5 groups) as follows: (1) the origin of VA occlusion (Origin group); (2) VA occlusion before branching into the PICA (Before group); (3) VA occlusion after branching into the PICA, which was divided into 2 groups—(3A) VA symptomatic occlusion after branching into the PICA (After group) and (3B) hypoplastic or asymptomatic occlusive VA after branching into the PICA (PICA end group); and (4) no significant occlusive lesions in the VAs (Control group). The After group was defined as symptomatic VA occlusion associated with acute ischemic stroke presented as a new infarct on MRI including diffusion-weighted imaging (DWI) or transient ischemic attack (TIA) in the vertebrobasilar circulation. The clinical diagnosis of stroke and TIA was made by the attendant physician from the result of MRI (DWI) and neurological findings. When VA occlusion was symptomatic, we identified it as the After group, even if the diameter of the target VA was smaller than that of the contralateral VA.

Using B-mode scans with color imaging and pulsed-Doppler, one investigator with no previous knowledge of the patients' clinical information including angiographic findings (K.S.) measured the flow velocities of both VAs within 48 hours before or after IA-DSA. We used a Sonos 5500 duplex color-coded ultrasonographic device (Philips) equipped with a 7.5-MHz transducer. First, we measured the diameter of the both VAs at the C3–4, C4–5, or C5–6 levels. Second, the flow velocities of the VAs were obtained between the transverse process at the C3–4, C4–5, or C5–6 levels of the cervical spine. The sample volume (2 to 3 mm, depending on the diameter of the VA) was set within the VAs and flow velocities were measured, taking care to maintain an adequate angle of ≤60 degrees between the beam and the VAs. The pulse repetition frequency was 3.0 or 3.5 Hz, and the low pass filter was set at 70 Hz. We obtained the peak systolic flow velocity (PSV), the end-diastolic flow velocity (EDV), and the time-averaged peak mean flow velocity (MV), corrected using the adequate angle for both VAs. Resistance index (RI) was defined as (PSV-EDV)÷PSV. The diameter-ratio (diameter of contralateral VA divided by diameter of target VA) and MV-ratio (MV of contralateral VA divided by that of target VA) were also determined. The diameter, diameter-ratio, and flow velocity data for each group were expressed as mean±SD.

Brain computed tomography (CT) and MRI including DWI were performed in all the patients to assess new brain infarctions. Conventional MRI T1-weighted (repetition time [TR]/echo time [TE]; 630/14), T2-weighted (TR/TE; 5400/99), and fluid-attenuation inversion recovery (FLAIR) (TR/TE; 9000/105) images were obtained. DWI was performed simultaneously using a spin-echo planar imaging sequence. Diffusion gradients were applied in the x, y, and z directions, with a b value of 1000/cm^2.

Statistical analysis was performed using the Mann-Whitney U test and Kruskal-Wallis test. A value of $P<0.05$ was accepted as indicating statistical significance. Sensitivity and specificity curves were produced to obtain the best cut-off value for each diagnostic criterion.

Results

The VAs were clearly displayed in all patients using B-mode with color imaging, and blood flow velocity was successfully evaluated by pulse Doppler (Figure 1). Table 1 shows the VA diameter, diameter-ratio, MV, MV-ratio, EDV, and RI of each VA.

Origin Group

Although the VAs were clearly detected using B-mode with color imaging, no blood flow signals, including MV and

TABLE 1. Parameters in Each Group

Group	N of Vessels	VA Diameter (mm)	Diameter-ratio	MV (cm/s)	MV-ratio	EDV (cm/s)	RI
Control	194	3.76±0.66	0.97±0.27	25.26±7.54	0.94±0.37	15.10±5.39	0.66±0.09
Origin	9	3.25±1.08	1.39±0.31	0	—	0	—
Before	10	3.25±0.72	1.20±0.42	7.24±4.64	2.38±1.55	0	1
After	12	3.37±0.64	1.10±0.23	12.92±3.29	2.00±0.80	6.69±3.74	0.78±0.14
PICA end	15	2.62±0.39	1.68±0.31	13.95±3.22	2.31±1.73	7.09±2.46	0.76±0.09
Total	240						

Diameter-ratio indicates diameter of contralateral VA divided by that of target VA; MV, mean flow velocity; MV-ratio, mean flow velocity of contralateral VA divided by that of target VA; EDV, end-diastolic flow velocity; RI, resistance index=(peak systolic flow velocity−end-diastolic flow velocity)÷peak systolic flow velocity.

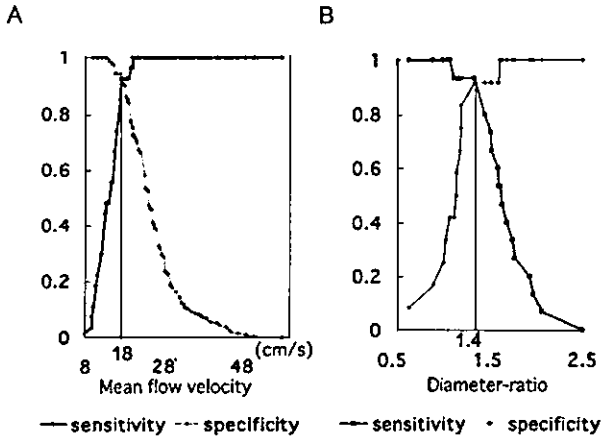

Figure 2. A, Sensitivity and specificity curve analysis for MV to discriminate the After and PICA-end groups from the Control group. B, Sensitivity and specificity curve analysis for diameter-ratio to discriminate the PICA end group from the After group.

EDV, in the VAs could be detected using pulse Doppler, allowing the Origin group VAs to be easily identified.

Before Group

Peak systolic flow velocity was preserved, but EDV was zero cm/s in all patients in the Before group. In addition, the MV (7.2±4.6 cm/s) was the lowest among all the groups, excluding the Origin group ($P<0.0001$). Excluding the Origin group, an EDV of zero cm/s allowed the Before group VAs to be easily distinguished from the other groups.

Distinguishing After and PICA End Groups From the Control Group

Of the 3 groups other than the Origin and Before groups, the MV, EDV, and RI of the After and PICA end groups (After group:12.9±3.3 cm/s, 6.7±3.7 cm/s, 0.78±0.14, respectively; PICA end group:14.0±3.2 cm/s, 7.1±2.5 cm/s, 0.76±0.09, respectively) were lower than those of the Control group (25.3±7.5 cm/s, 15.1±5.4 cm/s, 0.66±0.09, respectively) ($P<0.0001$). Using sensitivity–specificity curve analysis for discriminating the Control group from the After and PICA end group, the cut-off point of the RI and MV were 0.7 (sensitivity 74.0% and specificity 72.6%) and 18 cm/s (sensitivity 92.6% and specificity 90.2%; Figure 2A), respectively. Therefore, MV was a better parameter than RI for discriminating the After and PICA end groups from the Control group. However, 18 of 43 patients with MV <18 cm/s belonged to the Control group and the positive predictive value was low (58.1%). Of these 43 VAs with MV <18 cm/s, the sensitivity–specificity curve for MV-ratio to distinguish the After and PICA end groups from the Control group showed a cut-off value of 1.4 and gave a sensitivity of 84.0% and specificity of 82.3%. If we used the combined criteria of both MV <18 cm/s and MV-ratio ≥1.4 to distinguish the After and PICA end groups from the Control group, then sensitivity, specificity, accuracy, and positive predictive value were 85.2%, 97.4%, 95.9%, and 82.1%, respectively.

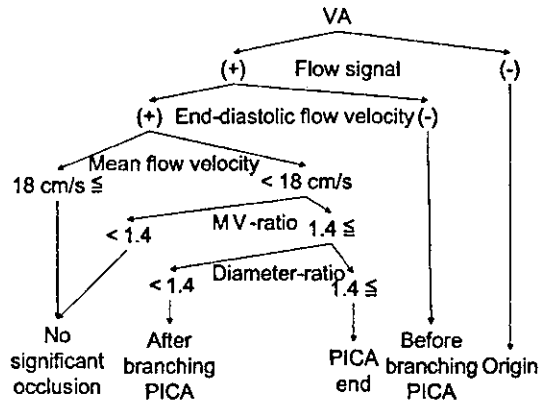

Figure 3. Ultrasonographic diagnostic algorithm for the site of VA occlusion.

Distinguishing the PICA End Group From the After Group

No significant difference in MV, EDV, and RI between the After group and PICA end group was observed. However, the diameter (2.62±0.39 mm) in the PICA end group was the smallest among all the groups (Before group: 3.25±0.72 mm; After group: 3.37±0.64 mm; Control group: 3.76±0.66 mm) ($P<0.0001$). The diameter-ratio (1.68±0.31) in the PICA end group was also the largest among all the groups (Before group: 1.20±0.42; After group: 1.10±0.23; Control group: 0.97±0.27, respectively) ($P<0.0001$). Using sensitivity–specificity curve analysis for discriminating the PICA end group from the After group, the cut-off point of VA diameter and diameter-ratio were 2.8 mm (sensitivity 73.3% and specificity 83.3%) and 1.4 (sensitivity 93.3% and specificity 91.7%), respectively (Figure 2B). Therefore, the diameter-ratio was a better parameter than VA diameter for discriminating the PICA end group from the After group.

Ultrasonographic Diagnostic Criteria

Figure 3 shows the criteria for the site of VA occlusion, including PICA end with duplex color-coded ultrasonography based on the present results. Table 2 shows the relationship between the cerebral angiographic findings and our ultrasonographic diagnosis. One VA vessel of the After group had the diameter-ratio ≥1.4. Therefore, we classified it as the PICA end, based on ultrasonographic criteria. The accuracy for conformity between them was 95.0%.

Discussion

The present study has established the ultrasonographic diagnostic criteria for determining the site of VA occlusion. Kimura et al[14] demonstrated the usefulness of measurement of VA flow velocity using duplex ultrasonography for the localization of the site of VA occlusion. They reported EDV of zero cm/s in a VA occlusion sited before branching into the PICA, which is consistent with the present findings. Furthermore, they described that the MV was significantly lower in a VA occlusion after branching into the PICA than in the nonocclusive VA group. However, accurate diagnostic criteria for differentiating these types were not established in their study.

TABLE 2. Comparison of Angiographic and Ultrasonographic Diagnoses

		Ultrasonographic Diagnosis					
		Control	Origin	Before	After	PICA End	Total
Angiographic Diagnosis	Control	189	0	0	3	2	194
	Origin	0	9	0	0	0	9
	Before	0	0	10	0	0	10
	After	2	0	0	9	1	12
	PICA end	2	0	1	1	11	15
	Total	193	9	11	13	14	240

In the present study, except for patients in the Origin and Before groups, 98.9% of patients with MV ≥18 cm/s had nonocclusive VAs, whereas 41.9% of patients with MV <18 cm/s also had nonocclusive VAs. Therefore, the criteria of threshold of MV <18 cm/s alone were insufficient to accurately distinguish the After group from the Control group. Using the combination of both MV-ratio ≥1.4 and MV <18 cm/s, sensitivity, specificity, accuracy, and positive predictive value to distinguish the After and PICA end groups from the Control group were much better at 85.2%, 97.4%, 95.9%, and 82.1%, respectively.

The VA blood flow wave and velocity between the After and PICA end groups were similar. Thus, we were unable to distinguish these groups by blood flow alone. Most hypoplastic VAs end in the PICA, and hypoplastic VA has been defined as a VA diameter of <2 mm.[13,23–25] In the present study, the mean and range of VA diameter in the PICA end group were certainly small, at 2.62±0.39 mm and 1.70 to 3.14 mm, respectively, and the PICA end group diameter was the smallest among the 5 groups. Therefore, the hypoplastic VA criteria of <2 mm may be high in specificity but low in sensitivity. When we used a cut-off value of 2.8 mm obtained from sensitivity and specificity curve analysis to distinguish the PICA end group from the After group, the accuracy was 77.8%, which was not overly useful. However, when we used a diameter-ratio ≥1.4 for the analysis, the sensitivity, specificity, and accuracy increased to 93.3%, 91.7%, and 92.6%, respectively, which was superior to that obtained using a cut-off VA diameter value of 2.8 mm. Therefore, a diameter-ratio ≥1.4 was identified as the criterion with which to differentiate between the After and PICA end groups. Diameter-ratio of symptomatic VAs occlusion was usually <1.4. In this study, however, we had 1 symptomatic VA occlusion with the diameter-ratio ≥1.4, which was diagnosed as PICA end by ultrasonography. This point may be one of the limitations in the present study.

Nicolau et al[13] examined RI in VA occlusion but did not discriminate the site of VA occlusion between before and after branching into the PICA. They reported that the RI in VA occlusion was higher than in non-VA occlusion. In the present study, although RI was higher in the After group than in the other groups, MV was superior to RI as a parameter to determine VA occlusion.

In the present study, the MV in 15 (18 vessels) of 117 patients in the Control group was <18 cm/s. Of these 15, 3 had an occlusion at the top of the basilar artery (BA), and 3 had bilateral fetal type of the posterior cerebral arteries (PCA). The blood flow of the VAs may be decreased under such conditions. This finding represents a limitation to the use of our criteria for identification of VA occlusion site.

We did not have any stenotic VAs in our present study. When the origin of VAs had stenosis, the blood flow velocity sometime reduces. Bray et al[26] reported that the velocity curve of severe stenotic VAs with their origin showed isolated ascending and lengthened systolic time and a systolic notch. Therefore, we should be able to distinguish it from the distal VA occlusion.

Another limitation is that asymptomatic acquired VA occlusion cannot always be distinguished from naturally hypoplastic VA ending in the PICA, as differentiating them is difficult in some patients, even with the findings of IA-DSA, MRI, and clinical symptoms. Therefore, the PICA end group may include asymptomatic acquired VA occlusion. In addition, in the present study, there were no patients with bilateral VA occlusion after branching into the PICA. Such patients may have been erroneously assigned into the Control group, because the MV-ratio in patients with bilateral VA occlusion would have been <1.4. Therefore, if a patient's neurological findings suggest occlusive lesions of the BA or VA after branching into the PICA, and the MV of both VAs is <18 cm/s and the MV-ratio is <1.4, those vessels would need to be assessed by transcranial Doppler or transcranial color-coded sonography.

In conclusion, measurement of the blood flow velocity and diameter of the VAs using duplex color-coded ultrasonography can help diagnose the site of VA occlusion. Ultrasonography is a noninvasive tool and can be performed bedside immediately after stroke patient admission. The present VA occlusion criteria may be used to evaluate VA occlusive lesions in acute stroke patients, in particular, those with medullary and brain stem infarction.

References

1. Moneta GL, Edwards JM, Chitwood RW, Taylor LM Jr, Lee RW, Cummings CA, Porter JM. Correlation of North Am Symptomatic Carotid Endarterectomy Trial (NASCET) angiographic definition of 70% to 99% internal carotid artery stenosis with duplex scanning. *J Vasc Surg.* 1993;17:152–159.
2. Kimura K, Yasaka M, Minematsu K, Wada K, Uchino M, Yonemura K, Ogata J, Yamaguchi T. Oscillating thromboemboli within the extracranial internal carotid artery demonstrated by ultrasonography in patients with acute cardioembolic stroke. *Ultrasound Med Biol.* 1998;24:1121–1124.
3. Kimura K, Yonemura K, Terasaki T, Hashimoto Y, Uchino M. Duplex carotid sonography in distinguishing acute unilateral atherothrombotic from cardioembolic carotid artery occlusion. *AJNR Am J Neuroradiol.* 1997;18:1447–1452.

4. Wada K, Kimura K, Minematsu K, Yasaka M, Uchino M, Yamaguchi T. Combined carotid and transcranial color-coded sonography in acute ischemic stroke. *Eur J Ultrasound.* 2002;15:101–108.
5. Yasaka M, Omae T, Tsuchiya T, Yamaguchi T. Ultrasonic evaluation of the site of carotid axis occlusion in patients with acute cardioembolic stroke. *Stroke.* 1992;23:420–422.
6. Wolverson MK, Heiberg E, Sundaram M, Tantanasirviongse S, Shields JB. Carotid atherosclerosis: high-resolution real-time sonography correlated with angiography. *AJR Am J Roentgenol.* 1983;140:355–361.
7. Trattnig S, Hubsch P, Schuster H, Polzleitner D. Color-coded Doppler imaging of normal vertebral arteries. *Stroke.* 1990;21:1222–1225.
8. Trattnig S, Schwaighofer B, Hubsch P, Schwarz M, Kainberger F. Color-coded Doppler sonography of vertebral arteries. *J Ultrasound Med.* 1991;10:221–226.
9. Bendick PJ, Jackson VP. Evaluation of the vertebral arteries with duplex sonography. *J Vasc Surg.* 1986;3:523–530.
10. Bartels E, Fuchs HH, Flugel KA. Duplex ultrasonography of vertebral arteries: examination, technique, normal values, and clinical applications. *Angiology.* 1992;43:169–180.
11. Davis PC, Nilsen B, Braun IF, Hoffman JC Jr. A prospective comparison of duplex sonography vs angiography of the vertebral arteries. *AJNR Am J Neuroradiol.* 1986;7:1059–1064.
12. Kimura K, Yamaguchi T, Yasaka M, Tsuchiya T. [Hemodynamics of the vertebral artery in subclavian steal syndrome and subclavian steal phenomenon]. *Rinsho Shinkeigaku.* 1991;31:970–973.
13. Nicolau C, Gilabert R, Chamorro A, Vazquez F, Bargallo N, Bru C. Doppler sonography of the intertransverse segment of the vertebral artery. *J Ultrasound Med.* 2000;19:47–53.
14. Kimura K, Yasaka M, Moriyasu H, Tsuchiya T, Yamaguchi T. Ultrasonographic evaluation of vertebral artery to detect vertebrobasilar axis occlusion. *Stroke.* 1994;25:1006–1009.
15. Hennerici M, Klemm C, Rautenberg W. The subclavian steal phenomenon: a common vascular disorder with rare neurologic deficits. *Neurology.* 1988;38:669–673.
16. Yip PK, Liu HM, Hwang BS, Chen RC. Subclavian steal phenomenon: a correlation between duplex sonographic and angiographic findings. *Neuroradiology.* 1992;34:279–282.
17. Kliewer MA, Hertzberg BS, Kim DH, Bowie JD, Courneya DL, Carroll BA. Vertebral artery Doppler waveform changes indicating subclavian steal physiology. *AJR Am J Roentgenol.* 2000;174:815–819.
18. Touboul PJ, Mas JL, Bousser MG, Laplane D. Duplex scanning in extracranial vertebral artery dissection. *Stroke.* 1988;19:116–121.
19. Bartels E, Flugel KA. Evaluation of extracranial vertebral artery dissection with duplex color-flow imaging. *Stroke.* 1996;27:290–295.
20. Hoffmann M, Sacco RL, Chan S, Mohr JP. Noninvasive detection of vertebral artery dissection. *Stroke.* 1993;24:815–819.
21. Sturzenegger M, Mattle HP, Rivoir A, Ribs F, Schmid C. Ultrasound findings in spontaneous extracranial vertebral artery dissection. *Stroke.* 1993;24:1910–1921.
22. Osborn AG. The vertebrobasilar system. In: Osborn AG, ed. *Cerebral angiography.* 2nd ed. Philadelphia: Lippincott Williams &Wilkins; 1999: 173–194.
23. Delcker A, Diener HC. [Various ultrasound methods for studying the vertebral artery—a comparative evaluation]. *Ultraschall Med.* 1992;13: 213–220.
24. Tegeler CH, Babikian VL, Gomez CR. Vertebral sonography. *Neurosonology.* 1996;91–92.
25. Fisher CM, Gore I, Okabe N, White PD. Atherosclerosis of the carotid and vertebral arteries—extracranial and intracranial. *J Neuropathol Exp Neurol.* 1965;24:455–476.
26. de Bray JM, Pasco A, Tranquart F, Papon X, Alecu C, Giraudeau B, Dubas F, Emile J. Accuracy of color-Doppler in the quantification of proximal vertebral artery stenoses. *Cerebrovasc Dis.* 2001;11:335–340.

Neuronal cyclooxygenase-2 expression during spreading depression and focal brain ischemia

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Abstract

In order to clarify the pathophysiology of ischemic stroke, we examined a primate model eliciting SD, a primate thromboembolic model, and a rat model of focal brain ischemia. Immediately after the first SD, focal cortical hyperemia was demonstrated without being followed by spreading or persistent hypoperfusion. Cyclooxygenase-2 (COX-2) induction was detected in SD monkeys by microarray analysis. Immunoreactive neurons were observed in SD animals. In the thromboembolic model, upregulation of COX-2 mRNA expression was observed after 2 h of ischemia, but disappeared by 24 h in the ischemic core. In peri-infarct areas, where flow-metabolism uncoupling was observed, COX-2 expression persisted even after 24 h of ischemia. In focal ischemic rats, diffuse, neuronal COX-2 staining was found in peri-infarct areas as well as in discrete, immunoreactive neurons in the ischemic core. Robust increases in prostaglandin E_2 levels in the peri-infarct areas were demonstrated following 24 h of ischemia. In conclusion, neuronal COX-2 induction was observed in SD animals as well as within potentially viable hypoperfused brain areas. COX-2 expression and prostaglandin production in ischemic tissues depended on the degree and duration of the reduction in cerebral blood flow.

Key words: spreading depression, focal brain ischemia, cyclooxygenase 2, cerebral blood flow

1. Introduction

Cortical spreading depression (SD)[1] has been suggested to play a significant role in the development of ischemic injury under conditions of focal brain ischemia in rat models[2,3]. As proposed by the Stroke Therapy Academic Industry Roundtable[4], nonhuman primate studies are required to clarify the pathophysiology of ischemic stroke, and to verify the safety and efficacy of newly developed drugs that show promising results in rodents. In order to investigate the pathophysiology of acute ischemic stroke, we have developed a primate model eliciting SD and a primate thromboembolic stroke model.

Cyclooxygenase-2 (COX-2), a rate-limiting en-

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zyme in prostaglandin synthesis, was induced associated with either eliciting SD or focal brain ischemia in the cortex ipsilateral to the SD elicitation or brain ischemia in the nonprimate cortex[5]. Therefore we examined COX-2 expression and its reaction products during SD and focal brain ischemia in primates as well as rats.

All procedures in this study were approved by our Institutional Animal Research Committee and were performed in accordance with the standards published by the National Research Council (Guide for the Care and Use of Laboratory Animals).

2. Material and methods

2. 1. Spreading depression in a primate model

We used nine adult, male cynomolgus monkeys. Animals were anesthetized with pentobarbital (0.1 mg/kg, i.p.). Anesthesia was maintained with a N_2O/O_2 (70% : 30%) gas mixture inhalation under artificial ventilation through an experimental period. They were divided into 2 groups, such as normal control (group C, n = 3) and SD evoked animals (group SD, n = 6).

SD was elicited by applying 3.3 mol/L potassium chloride (KCl) through a burr hole made in the left parietal skull[6]. Two other burr holes were made rostral to the hole for KCl application. DC potentials were monitored with microelectrodes inserted into the cortex to a depth of 1 mm through the burr holes except the hole for KCl application.

Cerebral blood flow (CBF) was measured with PET and the ^{15}O-labeled water bolus injection method. A baseline CBF measurement was done once prior to application of KCl solution. CBF measurements were repeated 5 times, beginning 3 minutes after the first SD at intervals of approximately 15 minutes. After completion of the PET studies (at 120 min after KCl application), the brain tissues in the group SD were quickly removed after exsanguination following perfusion with cold saline. Samples of brain tissues in the group C were also obtained as the same manner as those in the group SD. We investigated the gene expression profile associated with SD by a cDNA array system containing 9,182 human elements, which was confirmed by RNA blot, immunoblot, and immunohistochemical analyses[7].

2. 2. Thromboembolic stroke model in primates

Thromboembolic stroke was produced in male cynomolgus monkeys (n = 4) as described previously[8]. CBF was measured with ^{15}O-labeled water before and 1, 2, 4, 6, and 24 hours after embolization. Cerebral glucose metabolic rate (CMRglc) was measured with [^{18}F] FDG methods 24 hours after embolization[9]. Lesion size and location 24 hours after embolization was determined by the 2, 3, 5-triphenyl-tetrazolium chloride (TTC) staining method.

For biochemical analyses for brain tissues in thromboembolic stroke model, we used 9 adult male cynomolgus monkeys ; 3 monkeys were served as normal control and the remaining 6 were as ischemic animals[10]. Two hours after a single autologous blood clot injection in 3 monkeys or after the completion of the PET studies in the other monkeys with 24 h-ischemia, brain tissues were perfused with cold saline and the animals were sacrificed. Three normal controls were also sacrificed as the same manner. Expression ratios of COX-2 mRNA were calculated as ratios of COX-2 mRNA against those of normal brains. Cell injury was evaluated by incorporation of digoxigenin deoxy-uridine-5'-triphosphate (dUTP) with the use of DNA polymerase I.

2. 3. Focal brain ischemia in rats

Male Sprague-Dawley rats (300-350 g, n = 40) were used. Focal brain ischemia was produced by the intraluminad occlusion of the ostium of the right middle cerebral artery with nylon monofila-

ments, as previously described[11]. Rats were sacrificed at time 0 and at different times points after arterial occlusion (1, 2, 3, 4, 6, 8, 12, and 24 h, n = 4-5/time point) and their brains immediately immersed in ice-cold saline. Several blocks were frozen in isopentane-dry ice and stored at −80℃ until use, whereas others were embedded in paraffin for immunohistochemistry. Analysis of COX-2 expression (mRNA, protein), and measurement of the concentrations of PGE_2 and the prostacyclin metabolite, 6-keto-$PGF_{1\alpha}$ in the peri-infarct areas and the ischemic core were performed. In some animals, N-isoproryl-*p*-[^{125}I]-iodoamphetamine ([^{125}I] IMP) (2.22 MBq/kg body weight) was injected into the femoral vein 5 min before sacrifice and *ex-vivo* autoradiography was performed to measure cerebral blood flow (CBF) as described previously[12].

3. Results

3. 1. SD in primates

SD waves were recorded in eight of the 9 monkeys. Single episode in three monkeys, twice in two, and six episodes in one were recorded in the rostral sites. In two of three animals with the caudal hole, one had eight episodes and another did once in the caudal sites for chemical stimulation while they did no SD waves in the rostral sites. The remaining one had twice episodes in the rostral and six episodes in the caudal sites. Focal hyperemia was demonstrated adjacent to the site of KCl application immediately after the first SD. Average cortical CBF in the ipsilateral hemisphere increased significantly immediately after the chemical stimulation ($p < 0.05$ by paired t-test), and the significant increase in CBF persisted throughout the experimental period of 2 hours. In the contralateral hemisphere, no significant changes in CBF were observed.

As a result of microarray analysis, increases in normalized signals of gene expression above 1.5-fold was cyclooxygenase-2 (COX-2) gene (1.6-fold), and signal levels in 265 genes were different by at least 1.3-fold between the 2 groups. COX-2 induction was confirmed by RNA blot, immunoblot, and immunohistochemical analyses. Intense immunoreactive neurons were induced in the animals with SDs.

3. 2. Focal brain ischemia in primates

CBF in the temporal cortex and the basal ganglia decreased to < 40% of the contralateral values 1 hour after embolization, following further decline in CBF as well as CMRglc at 24 hour of ischemia. These regions were consistently unstained with TTC, being indicated that both temporal cortex and basal ganglia ipsilateral to the arterial embolization were regarded as the ischemic core. While CBF was > 40% of the contralateral values 1 hour after the embolization and recovered gradually with time in the parietal cortex ipsilateral to the embolization. No obvious TTC-unstained lesions were demonstrated in these regions, implicated that the parietal cortex ipsilateral to the embolization was regarded as the ischemic penumbra. Increased in CMRglc at 24 hours of ischemia compared with those in the contralateral regions, an uncoupling of CBF and CMRglc, were demonstrated in these regions.

The upregulation of COX-2 mRNA expression was observed at 2 h (expression ratio was 7.4), but disappeared by 24 h in the ischemic temporal cortex, where cell injury was apparent by incorporation of dUTP. In the ischemic parietal cortex, where flow-metabolism uncoupling was observed, COX-2 mRNA was persistently induced even at 24 h after ischemia (expression ratio was 4.7), and few damaged cells could be detected by incorporation of dUTP as well as in each region from the hemisphere contralateral to the clot injection. Intense COX-2 immunoreactivity was found in discrete neurons in the ischemic parietal

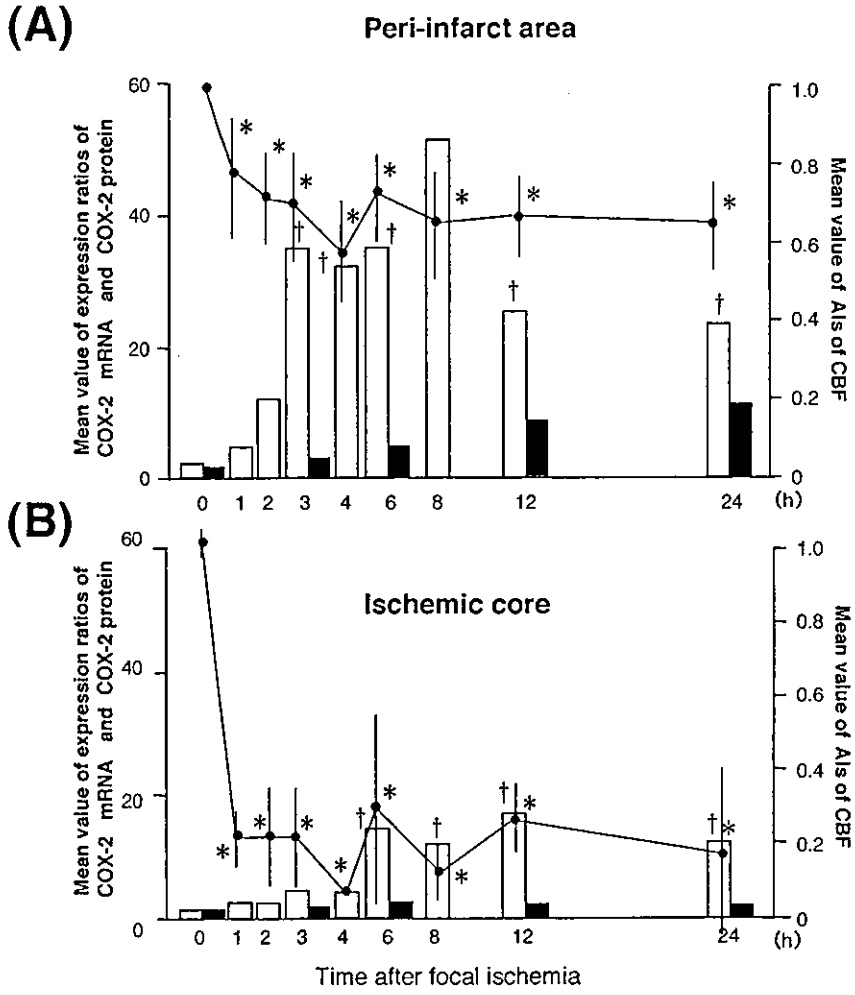

Fig. 1. Temporal profile of COX-2 expression associated with changes in CBF during 24 h of ischemia

Lines indicate the mean asymmetry index (AI) values of CBF. The open and solid columns correspond to the mean expression ratios of COX-2 mRNA and COX-2 protein, respectively. Figures A and B show the time course of COX-2 expression in the peri-infarct areas and ischemic core, respectively. A one-way ANOVA and post-hoc Fisher's tests were used to assess the differences in AIs and expression ratios of COX-2 mRNA between the different ischemic time points. CBF in the peri-infarct areas and ischemic core were significantly reduced compared to controls immediately after arterial occlusion (*: $p<0.05$). The mean CBFs in the ischemic core and peri-infarct areas were 0.19 ± 0.07 (mean ± SD) and 0.67 ± 0.06, respectively. The time course of COX-2 expression in the peri-infarct areas was different from that in the ischemic core. Thus, the expression ratios of COX-2 mRNA increased significantly after 3 h of ischemia (†: $p<0.05$), with COX-2 protein increasing with time in the peri-infarct areas. On the other hand, significant increases in COX-2 mRNA were found 6 h after ischemia (†: $p<0.05$), and increases in COX-2 protein were not observed during the ischemic period in the ischemic core.

cortex, although no significant increases in COX-2 protein level were shown either in the ischemic temporal or parietal cortices.

3. 3 Focal brain ischemia in rats

Table 1. Prostaglandin production (pg/mg total protein) in the ischemic hemisphere

Prostaglandin	Duration of ischemia	Peri-infarct area	Ischemic core
PG E_2	0 hours	60.8 ± 16.6	21.4 ± 11.4
	3 hours	156.6 ± 70.1	54.4 ± 22.3
	24 hours	2,609.0 ± 2,522.0 *†	414.6 ± 226.3 *†
Prostacyclin	0 hours	122.3 ± 47.6	47.6 ± 23.0
metabolite	3 hours	200.8 ± 59.7	93.4 ± 43.5
(6-keto-PG $F_{1\alpha}$)	24 hours	1,143.0 ± 623.7 *†	341.6 ± 84.5 *†

* $p < 0.05$ vs. 0 h (control) ; † : $p < 0.05$ vs. 3 h ischemia by ANOVA
The values were the mean ± SD.

Significant reductions in CBF in the peri-infarct areas and ischemic core were demonstrated in animals at each ischemic time point compared to the controls (Fig. 1). The expression ratios of COX-2 mRNA increased significantly between 3 and 24 h of ischemia in the peri-infarct areas compared to the controls. In the ischemic core, significant increases in COX-2 mRNA were seen following 6 h of ischemia, which remained through 24 h. The peak value of the expression ratio of COX-2 protein in the peri-infarct area was 10.7 at 24 h of ischemia, while the peak expression ratio in the ischemic core was 2.0 at 6 h of ischemia. COX-2 immunoreactive neurons were found predominantly in the peri-infarct area. Elevations in the immunohistochemical staining of discrete neuronal populations were also observed in the ischemic core. Although no significant ir creases in PGE_2 and prostacyclin levels were observed in the peri-infarct and ischemic core areas following 3 h of ischemia, significant increases in prostaglandin levels were found in the ischemic hemisphere following 24 h of ischemia. In particular, PGE_2 levels in the peri-infarct area increased significantly (Table 1).

4. Discussion

The CBF pattern obtained in the SD model of primates differed from those obtained in other studies using rat- and cat-SD models[13,14]. The focal hyperemia was not followed by prolonged hypoperfusion. The changes in CBF during SD phenomenon in primates also differed from those in patients with migraine[15]. In biochemical analysis for brain tissues, COX-2 was induced in the cortices where SD was recorded, being in accord with prvious observations in rodent models[5].

We observed COX-2 expression during focal brain ischemia in a primate thromboembolic stroke model. In the ischemic core, in which a signficnat decrease in CBF were accompanied by reduced CMRglc, upregurated COX-2 mRNA at 2 h-ischemia but decreased by 24 h. Disappearance of COX-2 at 24 h-ischemia was parallel to a housekeeping GAPDH-mRNA reduction, indicating that ischemic injury was already apparent at 24-ischemia in the temporal cortex and the basal ganglia. In the peri-infarct area, on the contrary, induced expression of COX-2 mRNA was still found at 24-h ischemia in the parietal cortex with a mild CBF reduction and maintained CMRglc.

In the focal ischemia in rats, the time course of COX-2 expression in the ischemic core was different from that seen in the peri-infarct area. The upregulation of COX-2 mRNA in the peri-infarct area persisted for at least 24 h after ischemia, as did the production of COX-2 protein, which lead

to significant increases in prostacyclin as well as PGE_2 levels following 24 hours of ischemia, though significant increases in COX-2 mRNA persisted during the 24 h of ischemia, though significant increases in COX-2 protein were not observed. This latter finding may be attributable to the severe ischemic injury that was caused by reduced CBF, which likely affected protein synthesis[2]. In spite of these effects on COX-2 protein, significant increases were seen in the concentration of prostaglandins in the ischemic core 24 hours after ischemia. Local increases in neuronal COX-2 expression in the ischemic core, as determined by immunohistochemical analysis, could have accounted for this increase in prostaglandin concentration.

The induction of neuronal COX-2 is important for the regulation of prostaglandin signaling in post-ischemic regions, and the magnitude of COX-2 activity and prostaglandin production is determined by the degree and duration of CBF reduction. Before novel therapeutic options for stroke patients can be developed, further clarification of the effects of COX-2 during and after ischemia will be required.

Acknowledgment

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References

1) Leao AAP : Spreading depression of activity in the cerebral cortex. J Neurophysiol 7 : 359—390, 1944
2) Hossmann KA : Viability thresholds and the penumbra of focal ischemia. Ann Neurol 36 : 557—565, 1994
3) Takano K, Latour LL, Formato JE, Carano RAD, Helmer KG, Hasegawa Y, Sotak CH, Fisher M : The role of spreading depression in focal ischemia evaluated by diffusion mapping. Ann Neurol 39 : 308—318, 1996
4) Stroke Therapy Academic Industry Roundtable : Recommendations for standards regarding preclinical neuroprotective and restorative drug development. Stroke 30 : 2752—2758, 1999
5) Miettinen S, Fusco FR, Yrjanheikki J, Keinanen R, Hirvonen T, Roivainen R, Narhi M, Hokfelt T, Koistinaho J : Spreading depression and focal brain ischemia induce cyclooxygenase-2 in cortical neurons through n-methyl-d-aspartic acid-receptors and phospholipase a_2. Proc Natl Acad Sci USA 94 : 6500—6505, 1997
6) Yokota C, Kuge Y, Hasegawa Y, Tagaya M, Abumiya T, Ejima N, Tamaki N, Yamaguchi T, Minematsu K : Unique profile of spreading depression in a primate model. J Cereb Blood Flow Metab 22 : 835—842, 2002
7) Yokota C, Inoue H, Kuge Y, Abumiya T, Tagaya M, Hasegawa Y, Ejima N, Tamaki N, Minematsu K : Cyclooxygenase-2 expression associated with spreading depression in a primate model. J Cereb Blood Flow Metab 23 : 395—398, 2003
8) Kito G, Nishimura A, Susumu T, Nagata R, Kuge Y, Yokota C, Minematsu K : Experimental thromboembolic stroke in cynomolgus monkey. J Neurosci Meth 105 : 45—53, 2001
9) Kuge Y, Yokota C, Tagaya M, Hasegawa Y, Nishimura A, Kito G, Tamaki N, Hashimoto N, Yamaguchi T, Minematsu K : Serial changes in cerebral blood flow and flow-metabolism uncoupling in primates with acute thromboembolic stroke. J Cereb Blood Flow Metab 21 : 202—210, 2001
10) Yokota C, Kuge Y, Inoue H, Tagaya M, Kito G, Susumu T, Tamaki N, Minematsu K : Post-ischemic cyclooxygenase-2 expression is regulated by the extent of cerebral blood flow reduction in non-human primates. Neurosci Lett 341 : 37—40, 2003
11) Minematsu K, Li L, Fisher M, Sotak CH, Davis MA, Fiandaca MS : Diffusion weighted magnetic resonance imaging : Rapid and quantitative detection of focal brain ischemia. Neurology 42 : 235—240, 1992
12) Yokota C, Kuge Y, Inoue H, Tamaki N, Minematsu K : Temporal and topographic profiles of cyclooxygenase-2 expression during 24 hours of focal brain ischemia in rats. Neurosci Lett 357 : 219—222, 2004
13) Lauritzen M, Jorgensen MB, Diemer NH, Gjedde

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REGULAR ARTICLE

Optimal dose of prothrombin complex concentrate for acute reversal of oral anticoagulation

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KEYWORDS
Hemorrhagic complication;
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Abstract We investigated optimal dose of prothrombin complex concentrate (PCC) for acute reversal of oral anticoagulation in patients with major hemorrhagic complications or who required invasive procedures. We also checked how rapidly international normalized ratio (INR) was reversed after PCC administration.

INR was measured before and 10–60 min after administration of PCC with or without vitamin K in 42 patients (men 28, women 14, median age of 70 years old) who had received warfarin but required rapid reversal of INR because of a hemorrhagic complication or medical procedure. The amount of PCC administered was 200 IU in six patients, 500 IU in 30, 1000 IU in 3, and 1500 IU in the other 3. Additional administration of PCC was performed when the correction of INR was inadequate. In 10 of the 42 cases, INR was measured serially, before, 10 and 60 min and 12–24 h after the administration of PCC and vitamin K.

In the six patients who received PCC of 200 IU, INR values of 3.34 median (range 2.06 to 5.08) decreased to 1.85 (range 1.23 to 2.43) significantly (Wilcoxon's rank sum test, $p=0.028$), but in three patients (50%), INR values were still above 2.0 after the administration. In 30 patients treated with PCC of 500 IU, values decreased from 2.49 median (range 1.54 to 10.00) to 1.19 (range 0.87 to 1.55) significantly ($p<0.0001$). The corrected INR values were below 1.5 in 25 of 26 patients (96%) who had initial INR values from 2.0 to 4.9. In four patients with initial INR of 5.0 or more, the reversed INR was below 1.5 in one (25%), between 1.5 and 2.0 in two (50%), and above 2.0 in one (25%) who had additional administration of 500 IU PCC lowering INR from 2.01 to 1.48. Values of INR in the six patients receiving 1000 IU or 1500 IU, INR decreased from 2.33 median (range 1.96 to 4.00) to 0.96 (range 0.87 to 1.24, $p=0.028$).

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In the 10 patients with serial measurement, INR changed from 2.67 median (range 2.05 to 10.00) to 1.17 (range 0.99 to 1.60) 10 min after the administration. The INR values remained stable 60 min and 12–24 h after the PCC administration.

The 500 IU of PCC is likely to be optimal dose of PCC for emergent reversal of INR in patients requiring rapid correction of INR below 5.0, but to be inadequate dose in patients with INR of 5.0 or more. PCC administration with vitamin K may finish reversing INR rapidly within 10 min and keep the reversed INR values for 12–24 h.

Introduction

Hemorrhagic complication is a major adverse events in patients with oral anticoagulant therapy [1,2], and often requires reduction in dose or discontinuation of the therapy, administration of vitamin K, fresh frozen plasma (FFP) or prothrombin complex concentrate (PCC). The PCC contains coagulant factors II, VII, IX, and X and can reverse the effect of warfare more rapidly than FFP in warfarin-related coagulopathy [3–7]. We reported earlier that rapid correction of international normalized ratio (INR) prevented enlargement of intracranial hematoma in patients with INR values above 2.0 within 24 h of hemorrhagic stroke onset [8].

However, several questions remain to be resolved in the PCC treatment; how much PCC should be administered initially and how rapidly the PCC treatment can reverse INR. The present study was carried out to solve these questions.

Material and methods

From December 2000 to August 2003, PCC was administered in 42 patients who were given warfarin treatment, but required rapid correction of INR because of a major hemorrhagic complication or invasive procedures, an insertion of drainage tube into the thoracic cavity due to acute pneumothorax in two patients and an operation to remove a part of skull bone due to local infection. The major hemorrhagic complications were cerebral hemorrhage in 27, acute epidural hemorrhage in seven, acute bleeding from the gastrointestinal system in two, acute subdural hemorrhage, massive subcutaneous hemorrhage, and intramuscular hemorrhage in one each.

They were 28 men and 14 women with 24–90 years of age (median 70 years old). The underlying diseases requiring warfarin treatment were atrial fibrillation in 22, prosthetic cardiac valves in 9, deep vein thrombosis in 4, left ventricular assist systems in 2, Buerger disease, basilar stenosis, old myocardial infarction, dilated cardiomyopathy, and aortic arch atherosclerosis in 1 each. Hypertension, brain infarction, hypercholesterolemia, diabetes mellitus and hepatitis was complicated in 32 (76.2%), 24 (58.5%), 14 (34.1%), 8 (19.5), and 2 (4.8%) patients, respectively.

The pharmaceutical council in our hospital discussed the administration of PCC including ethical issue and approved it for emergent INR reversal after obtaining informed consent. Then written informed consent was always obtained from the patients or their family. For each patient, administration and amount of the PCC were decided by physicians in charge according to our previous studies [7,8]. The initial amount of PCC was 200 IU in 6, 500 IU in 30, 1000 IU in 3 and 1.500 IU in the other 3. We administered vitamin K of 10 mg in 20 patients and 20 mg in 11 with PCC. Additional PCC was given if the INR value was still high just after the first PCC administration. We used a commercially available PCC "PPSB-HT Nichiyaku" produced by Nihon Pharmaceutical, Tokyo, Japan, which contained 500 IU of II, VII, IX, X and 380 U of protein C in 25 ml. The PCC was derived from donated plasma, which was negative for HBs antigen, anti HCV antibody, anti HIV-1 antibody, anti HIV-2 antibody, anti-HTLV-I antibody and screened by ALT values. Heat at 65 C for 96 h and nanofiltration were applied to inactivate viruses. PCC was extracted from a bottle through a filter to an injection syringe and infused through a venous line in 5–10 min.

INR values were measured before and 10 to 60 min after administration of PCC with or without vitamin K in 42 patients. In 10 of the 42 cases, they were measured serially, before, 10 and 60 min and 12–24 h after the administration of PCC (500 IU in nine and 1000 in the other one) and vitamin K (10 mg in seven and 20 mg in the other three).

Data were expressed as median and range. We used Wilcoxon's rank sum test for analysis of

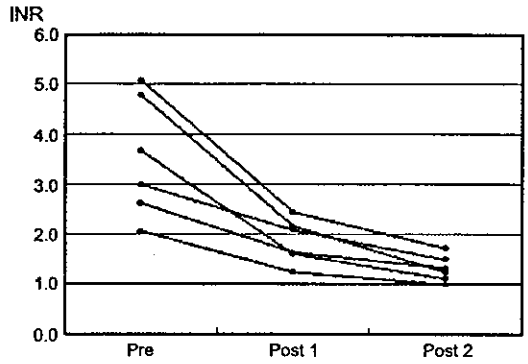

Figure 1 INR values before (Pre) and after PCC administration of 200 IU (Post 1), and those after additional administration of 300 IU (Post 2).

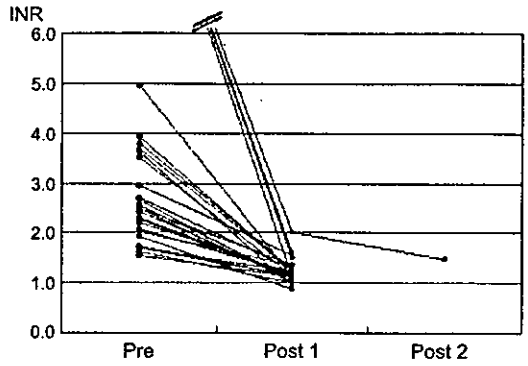

Figure 2 INR values before (Pre) and after PCC administration of 500 IU (Post 1), and those after additional administration of 500 IU (Post 2).

variables. A p-value less than 0.05 was considered significant.

Results

In six patients who received PCC of 200 IU (median 3.3 IU/kg, range 2.6 to 5.3 IU/kg), INR values decreased from 3.34 median (range 2.06 to 5.08) to 1.85 (range 1.23 to 2.43) significantly (p=0.028), but INR values after the PCC administration were still above 2.0 in three patients and between 1.5 and 2.0 in two patients (Fig. 1). Additional 300 IU was administered into the six patients, and INR values decreased to 1.28 (range 0.99 to 1.71).

In 30 patients treated with PCC of 500 IU (median 8.8 IU/kg, range 6.0 to 17.9 IU/kg), INR values decreased from 2.49 median (range 1.54 to 10.00) to 1.19 (range 0.87 to 1.55) significantly (p<0.0001, Fig. 2). INR values after PCC administration were below 1.5 in 25 of 26 patients (96%) who had initial INR values from 2.0 to 4.9 and between 1.5 and 2.0 in two (50%) and above 2.0 in the one (25%) of the four patients that had initial INR of 5.0 or more (Table 1). Only one patient with INR value of 2.01 after 500 IU of PCC administration received additional 500 IU of PCC (1000 IU in total) and his INR decreased to 1.48.

Values of INR in patients receiving 1000 IU (median 18.4 IU/kg, range 18.1 to 18.7 IU/kg) or 1500 IU (median 26.0 IU/kg, range 25.2 to 26.8 IU/kg), INR values decreased from 2.33 median (range 1.96 to 4.00) to 0.96 (range 0.87 to 1.24, p<0.028, Fig. 3).

In 10 patients with serial measurement, INR changed from 2.67 median (range 2.05 to 10.00) to 1.17 (range 0.99 to 1.60) 10 min after the administration (p=0.0051, Fig. 4). The INR values remained stable after 60 min and 12–24 h after the administration.

Symptoms did not deteriorate and hematoma volume did not enlarge in 25 patients of the 27 with cerebral hemorrhage. Deterioration of symptoms with enlargement of hematoma volume was noted in only two, one of whom re-increase of INR from 1.48 to 2.72 half a day after INR reversal by administration of 1000 IU of PCC without vitamin K, and the other one of whom systolic blood pressure after admission remained above 200 mm Hg while INR was kept low. Evacuation of hematoma in six patients with acute epidural hematoma and in a patient with acute subdural hematoma was successfully performed and easy hemostasis during operation was noted by neurosurgeons while a patient with severe epidural hematoma at admission died despite INR reversal. Insertion of drainage

Table 1 Reversed INR according to the initial INR

Amount of PCC administered initially (%)									
	200 IU			500 IU			1000 or 1500 IU		
Reversed INR	≧2.0	≧1.5 & <2.0	<1.5	≧2.0	≧1.5 & <2.0	<1.5	≧2.0	≧1.5 & <2.0	<1.5
Initial INR									
5.0–	0	0	0	1 (25)	2 (50)	1 (25)	0	0	0
3.0–4.9	3 (75)	1 (25)	0 (0)	0	0	6 (100)	0	0	1 (100)
2.0–2.9	0	1 (50)	1 (50)	0	1 (8)	12 (92)	0	0	3 (100)
1.5–1.9	0	0	0	0	0	7 (100)	0	0	2 (100)
									(%)

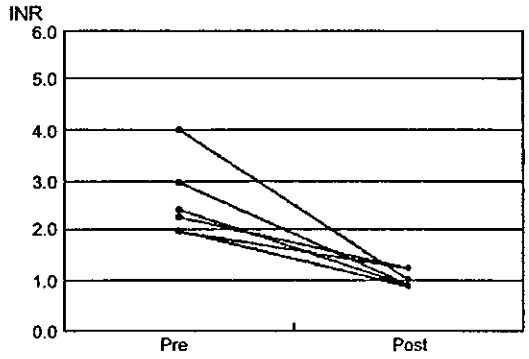

Figure 3 INR values before (Pre) and after PCC administration of 1000 or 1500 IU (Post).

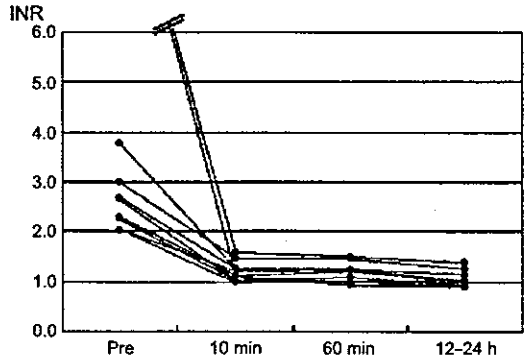

Figure 4 INR values before (Pre) and 10 min, 60 min, 12–24 h after PCC administration of 500 or 1000 IU. min: minutes, h: hours.

tube into the thoracic cavity and an operation to remove a part of skull bone were also successfully done. Subcutaneous, intramuscular and gastrointestinal bleeding stopped after the INR reversal by the PCC administration.

Any adverse effects including shock, allergy, or thrombotic or embolic episodes were not observed in the 42 patients.

Discussion

Previously we reviewed 47 patients on warfarin who developed acute intracerebral hematoma, 10 of whom had PCC treatment within 24 h of onset, and determined relationships among enlargement of the hematoma, INR reversal and clinical data [8]. Multivariate analysis showed an INR value <2.0 at admission or for 24 h after immediate INR reversal with PCC prevented the enlargement of hematoma. Fredriksson et al. retrospectively compared laboratory data and clinical features in 17 patients of anticoagulant-related intracerebral hemorrhage treated with PCC or FFP, and found that clinical progression within 12 h occurred in five of six patients with reversed INR of 1.46 or more [5]. Therefore, it seems that immediate reversal of INR and upkeep of INR values below 2.0 or below 1.5 is necessary to prevent progression of intracerebral hemorrhage.

Effect of 20–50 IU/kg of PCC on reversing INR was reported to be more rapid and effective than FFP [3–6]. Butler et al. [9] recommended administration of 50 IU/kg or 25 IU/kg of PCC with vitamin K in the immediate management of oral anticoagulant-related intracranial hemorrhage when INR values are >=4.5 or <4.5, respectively. The present study demonstrated that 500 IU (median 8.8 IU/kg, range 6.0 to 17.9) of PCC induced a rapid reversal of INR into below 1.5 in 96% of 26 patients who's initial INR values were below 5.0. Because, in the patients with INR above 5.0, INR values remained above 1.5 in 75% after the initial administration of 500 IU PCC and one of them had 1000 IU PCC in total to reverse INR fully, 500 IU PCC may be inadequate and initial administration of 1000 IU or 1500 IU PCC seem required to reverse INR fully in patients with INR above 5.0 (Tables 1 and 2).

Fredriksson et al. [5] reported bilateral renal infarction at autopsy in a case treated with 3000 IU PCC and noted the risk of general thromboembolism triggered by activated prothrombin complex. We did not find any adverse effects in patients treated with smaller amount of PCC (200–1500 IU) than in previous reports [3–6]. Therefore, the smaller amount of PCC might contribute to avoid

Table 2 Amount of PCC and INR change

Investigator	Number	PCC			VK (mg)	INR change	Time
		Amount (IU)	IU/kg	(median)			
Fredriksson et al. [[5]]	5		40–50		10	3.1–1.3	2 h
Makris et al. [[6]]	6		50		10	4.9–1.3	15 min
Boulis et al. [[3]]	10		25.8		10–20	2.8–1.2	4.8 h
Cartmil et al. [[4]]	16		20–50		1–5	5.8–1.3	15 min
Current study	6	200 IU	2.6–5.3	(3.3)	0–20	3.3–1.9	10–60 min
	30	500 IU	6.0–17.9	(8.8)	0–20	2.5–1.2	10–60 min
	3	1000 IU	18.1–18.7	(18.4)	0–10	2.3–1.0	10–60 min
	3	1500 IU	25.2–26.8	(26.0)	0–10	2.4–0.9	10–60 min

thrombotic or embolic adverse effects including disseminated intravascular coagulation. However, the present study showed that initial amount of 200 IU was so inadequate to reverse INR that we had better to administer 500 IU or more initially.

Correction of INR values was reported to be confirmed 15 min, 2 h, or 4.8 h after PCC administration [3–6]. Preston et al. [10] demonstrated rapid reversal of INR by measuring blood samples obtained at 20, 60, and 120 min after treatment. According to the present study, correction of INR seems to be accomplished more quickly, within 10 min after completion of PCC administration than indicated in the previous five reports. Excessive INR values may be counteracted immediately with increases of coagulant factors II, VII, IX, and X by the PCC administration.

Because the present study was not a randomized one, we need prospective randomized research to confirm optimal initial dose of the PCC according to INR.

In conclusion, 500 IU of the PCC is likely to be optimal for rapid correction of INR below 5.0 but to be inadequate in patients with INR of 5.0 or more. PCC administration with vitamin K may finish correcting INR rapidly within 10 min and keep the lower INR values for 12–24 h.

Acknowledgements

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References

[1] Wintzen AR, de Jonge H, Loeliger EA, Bots GT. The risk of intracerebral hemorrhage during oral anticoagulant treatment: A population study. *Ann Neurol* 1984;16:553–8.
[2] Kase CS, Robinson RK, Stein RW, DeWitt LD, Hier DB, Harp DL, et al. Anticoagulant-related intracerebral hemorrhage. *Neurology* 1985;35: 943–48.
[3] Boulis NM, Bobek MP, Schmaier A, Hoff JT. Use of factor IX complex in warfarin-related intracranial hemorrhage. *Neurosurgery* 1999;45:1113–9.
[4] Cartmill M, Dolan G, Byrne JL, Byrne PO. Prothrombin complex concentrate for oral anticoagulant reversal in neurosurgical emergency. *Br J Neurosurg* 2000;14:458–61.
[5] Fredriksson K, Norrving B, Stromblad LG. Emergency reversal of anticoagulation after intracerebral hemorrhage. *Stroke* 1992;23:972–7.
[6] Makris M, Graves M, Phillips WS, Kitchen S, Rosendaal FR, Preston EF. Emergency oral anticogulant reversal: The relative efficacy of infusions of fresh frozen plasma and clotting factor concentrate on correction of the coagulopathy. *Thromb Haemost* 1997;77:477–80.
[7] Yasaka M, Sakata T, Minematsu K, Naritomi H. Correction of INR by prothrombin complex concentrate and vitamin K in patients with warfarin related hemorrhagic complication. *Thromb Res* 2003;108:25–30.
[8] Yasaka M, Minematsu K, Naritomi H, Sakata T, Yamaguchi T. Predisposing factors for enlargement of intracerebral hemorrhage in patients treated with warfarin. *Thromb Haemost* 2003;89:278–83.
[9] Butler AC, Tait RC. Management of oral anticoagulant-induced intracranial haemorrhage. *Blood Rev* 1998;12: 35–44.
[10] Preston FE, Laidlow ST, Sampson B, Kitchen S. Rapid reversal of oral anticoagulation with warfarin by a prothrombin complex concentrate (Beriplex): efficacy and safety in 42 patients. *Br J Haematol* 2002;116:619–24.

口科誌 J. Jpn. Stomatol. Soc. 53(2) : 74~80, March, 2004

抗血栓療法施行患者の抜歯における出血管理に関する検討

Hemostatic management of tooth extraction in patients undergoing antithrombotic therapy

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Abstract

We examined hemostatic management for tooth extraction in Japanese patients undergoing antithrombotic therapy.

The subjects consisted of 57 patients aged from 18 to 91 years old. Forty patients received warfarin therapy including 15 receiving additional antiplatelet drugs. The remaining 17 patients received antiplatelet drugs (aspirin, tichlopidine hydrochloride and cilostazol). In patients receiving warfarin therapy, 19 patients were controlled in International Normalized Ratio (INR)<2.0, 14 in INR 2.0-2.5 and 7 in INR 2.5-3.0.

One-hundred and six teeth were extracted on 65 occasions. All teeth were extracted without reducing the usual antithrombotic therapy, oxidized cellulose was applied and suturing was performed for local hemostasis.

Three of 65 cases of tooth extraction showed postoperative hemorrhage (4.6%); two occurred in patients under warfarin therapy, with INR of 2.15 and 2.49, respectively. The other case was a patient who received both aspirin and tichlopidine hydrochloride. In these patients, because gingivitis, alveolar and gingival abscess were observed, postoperative hemorrhage appeared to be caused by local inflammation rather than antithrombotic therapy.

These results suggest that sufficient hemostasis can be obtained in most cases of tooth extraction under anticoagulant therapy with warfarin (INR<3.0) and antiplatelet drugs. An appropriate local hemostatic method can obtain hemostasis in case of postoperative hemorrhage.

Key words : Antithrombotic therapy (抗血栓療法), Warfarin (ワルファリン), Antiplatelet drug (抗血小板薬), Tooth extraction (抜歯), Local hemostasis (局所止血)

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緒　言

近年，欧米では抗血栓療法施行患者の抜歯を，抗凝固療法薬であるワルファリンまたは抗血小板薬を中止・減量することなく維持量投与下に行うことが推奨されている。特にワルファリンについては，International Normalized Ratio（INR）が3.0以下（報告によっては4.0以下）であれば，維持量投与下の抜歯でも，中止した場合と比べて，後出血の発生率に差はみられないとの報告[1-13]が多い。

一方，日本においては，抜歯の際に抗血栓療法を，その程度にかかわらず中止・減量することが慣習化されており，それに伴う血栓症の報告[14]もみられる。しかし，抗血栓療法を受けている日本人の抜歯における出血管理に関する研究は数少ない。また，抗血栓療法の効果は欧米人と日本人とでは異なっているといわれているため，欧米の抜歯時の基準をそのまま日本人にあてはめることはできない。

そこで，本研究では，抗血栓療法を受けている日本人の抜歯の出血管理を調査し，適切な基準について検討した。

研究対象および方法

1.　対象症例

対象は，2002年4月～2003年9月の間に，大阪大学歯学部附属病院歯科麻酔科および国立循環器病センター歯科において抜歯を受けた抗血栓療法施行患者57名である。これらの患者の患者背景（性別，年齢，基礎疾患および歯科疾患の種類），抗血栓療法の使用薬剤と投与量，ワルファリン投与患者については抗血栓効果の程度，抜歯の内容，術後出血の有無とその内容を診療録から調査した。

対象患者57名のうち，男性35名，女性22名で，年齢分布は18～91歳であった。

基礎疾患としては，心筋梗塞20名，脳梗塞18名，心房細動15名，心筋症11名，人工弁置換術後10名，高血圧症4名，原発性肺高血圧症3名などであった（重複あり）。歯科疾患としては，辺縁性歯周炎48歯（うち歯肉膿瘍7歯），根尖性歯周炎52歯（うち埋伏した残根9歯，歯槽膿瘍1歯，歯根嚢胞2個），智歯周囲炎6歯であった（表1～4）。

ワルファリン投与患者は40名で，ワルファリンの投与量は1～4 mg/日であった。そのうち15名は抗血小板薬を併用していた。抗血小板薬のみの投与患者は17名で，投与量は，アスピリンは81～300mg/日，塩酸チクロピジンおよびシロスタゾールはそれぞれ200mg/日であった（表1～4）。

抗血栓効果の程度は，ワルファリン使用例では，抜歯当日に測定したプロトロンビン（PT）活性およびINRにて評価した。ただし，症例29の2回目の抜歯は，1回目の抜歯の1週間後に行ったものであるが，当日の凝血学的検査は行われていなかった。

2.　抜歯と局所出血管理

ワルファリンおよび抗血小板薬は，維持量を継続して抜歯を行った。抜歯後の局所止血法としては，萌出歯の抜歯では，抜歯窩に酸化セルロース綿（サージセル綿™）を挿入し，脱落防止のために4-0絹糸で1糸水平マットレス縫合した。また必要に応じて止血床を使用した。埋伏歯の抜歯では，抜歯窩に酸化セルロース綿を挿入し，創は4-0絹糸で縫合閉鎖した。他の止血剤は使用しなかった。

3.　出血の程度の判定

出血の判定は新美らの基準[15]を用い，抜歯創からの出血を，抜歯直後（抜歯約30分後）から1週間後まで評価した。出血の程度は，出血なし（Grade 0），湧出性の出血（Grade 1），顕著な出血（Grade 2）とし，Grade 1および2を後出血ありとした。

結　果

抜歯はのべ65回，合計106歯に行った。そのうち萌出歯は91歯，埋伏歯は15歯であった。後出血は，Grade 1が3回（4.6%）認められたが，Grade 2はみられなかった。

ワルファリン投与患者のうち，INRが2.0未満の19名において，21回（萌出歯32歯，埋伏歯5歯）の抜歯を行い，後出血は1回もみられなかった（表1）。

INRが2.0～2.5未満の14名において，16回（萌出歯18歯，埋伏歯3歯）の抜歯および嚢胞摘出術1回を行い，2回に後出血（Grade 1）を認めた（表2）。

症例19は，ワルファリン1.0mg/日の投与で，INRは2.15であった。$\overline{65}$|は根尖性歯周炎による歯槽膿瘍を伴っていたため，抗菌薬を投与し，急性炎症がやや軽減した後に，抜歯をした。しかし，抜歯2日後から出血がみられ，ガーゼによる圧迫のみでは止血が得られず，出血をくりかえし，7日後には抜歯窩には血腫が認められた。抜歯窩を再掻爬後に酸化セルロース綿を挿入し，1糸縫合し止血がえられた。症例30は，ワルファリン2.5mg/日の投与で，INRは2.49であった。抜歯した$\underline{3}$|は辺縁性歯周炎による歯肉発赤を伴っていた。抜歯後，酸化セルロース綿の挿入および縫合のうえ，歯周包帯（COE-PAK™）を用いて義歯を装着した。しかし，抜歯6日後から間欠的に出血をきたし，7日後には抜歯窩から持続的な湧出性出血を認めた。この時点で，INR 3.5とワルファリンの効果が増強していたため，ワルファリンを一時中断のうえ，抜歯窩を再掻爬し，酸化セルロース綿を挿入し，縫合して止血がえられた。

表1 INR 2.0未満の症例

症例	性別	年齢	基礎疾患名	歯科疾患名	歯科処置	抗凝固療法（/日） ワルファリン（mg）	アスピリン（mg）	シロスタゾール（mg）	INR	PT（%）	後出血	備考
1	M	63	CI, DM	1|23 P	抜歯	3.0	100		1.33	74		
				32|P 32|	抜歯	3.0	100		1.15	59		
2	F	46	PPH	|5 per 7|	抜歯	2.5			1.29	60		
3	M	35	AVR	8| *perico*	埋伏抜歯	2.0			1.29	60		
4	F	58	MVS	|6 per	抜歯	3.5			1.57	48		
5	F	48	AVR, TAA Marfan Synd	6| per 7|	抜歯	2.5			1.68	44		
				|457 per	抜歯	2.5			1.52	50		
6	M	78	MVR	7| P, GA	抜歯	2.5			1.80	39		
7	F	56	Af, CHF	4| P	抜歯	1.0	100		1.52	46		
8	M	89	OMI, CI	87| P	抜歯	3.0			1.80	39		
9	M	70	AMI, CI, HT, ASO	6| per	抜歯	1.0	81	200	1.60	43		
10	M	60	OMI	|35 per	抜歯	3.0	81		1.55	48		
11	M	82	Af, CI	1|1 P	抜歯	3.0			1.83	38		
12	M	58	HT, CI	|6 P	抜歯	2.0	200		1.65	44		
13	F	73	CI, Vf, SSS, MVR	|1 per, |45 P	抜歯	4.0			1.77	40		
14	F	18	DCM, PPH	|4 *per RR*	埋伏抜歯	2.5			1.59	49		
15	M	53	DCM, CHF, DM	|7 per	抜歯	2.0			1.70	36		
16	M	35	DCM, Af, CHF	8| *perico*	埋伏抜歯	1.5			1.74	41		
17	M	11	TOF	|C per	抜歯	1.4			1.20	60		
18	M	26	HCM, OMI	8| *perico*	埋伏抜歯	3.0	81		1.82	38		
19	M	75	OMI, Af, CI	3| *per RR*, 21| P	埋伏抜歯，抜歯	4.0			1.90	35		

P：辺縁性歯周炎，GA：歯肉膿瘍，per：根尖性歯周炎，perico：智歯周囲炎，RP：埋伏残根，italics：埋伏歯，
AMI：急性心筋梗塞，OMI：陳旧性心筋梗塞，AVR：大動脈弁閉鎖不全，MVR：僧房弁閉鎖不全，MVS：僧房弁狭窄，
CI：脳梗塞，Af：心房細動，DCM：拡張型心筋症，HCM：肥大型心筋症，HT：高血圧症，DM：糖尿病，
PPH：原発性肺高血圧症，TAA：胸部大動脈瘤，ASO：動脈閉塞症，CHF：慢性心不全，SSS：洞不全症候群，
Marfan synd：マルファン症候群，Vf：心室細動，TOF：ファロー四徴

INRが2.5～3.0未満の7名において，9回（萌出歯17歯，埋伏歯1歯）の抜歯および嚢胞摘出術1回を行い，後出血はみられなかった（表3）。

ワルファリン投与患者で後出血をきたしたのは，46回の抜歯のうち2回（4.4%）であった。

抗血小板薬のみを投与されていた患者17名では，抜歯を19回（萌出歯24歯，埋伏歯6歯）行い，後出血を1回（5.3%）認めた（表4）。後出血をきたした症例40は，アスピリン81mg/日およびシロスタゾール200mg/日の投与をうけていた85歳の患者で，抜歯した5432|は，辺縁性歯周炎による歯肉膿瘍を伴っていた。抗菌薬の投与により急性炎症がやや軽減した後に，抜歯を施行した。抜歯翌日から間欠的な出血を認め，7日後には抜歯窩に小血腫を認めたが，ガーゼによる圧迫のみで止血した。

考　察

現在，国際的には，ワルファリンの抗凝固作用を評価する基準として，International Normalized Ratio（INR）が用いられる。これは，各社の市販トロンボプラスチン試薬にて測定したプロトロンビン（PT）比を，WHOが標準品としたヒト脳トロンボプラスチンを用いた場合のPT比に換算した値として示される。この各社の試薬には，標準品との活性を比較して得られた指数がつけられており，これをInternational Sensitivity Index（ISI）と称する。