Table 3. Multiple regression analysis between CAVI and other clinical variables

Variable	Coefficiency	95% confidence interval	*p* value
Age	0.065	0.059-0.072	<0.0001
Gender	−0.045	−0.045-0.137	0.63
LDL cholesterol	0.0003	−0.002-0.003	0.83
Hemoglobin A1c	0.175	0.058-0.291	<0.01
Systolic BP	0.012	0.008-0.016	<0.0001
Smoking history	0.318	0.134-0.503	<0.001
eGFR	−0.005	−0.010--0.00001	<0.05

CI, confidence interval; LDL, low density lipoprotein; BP, blood pressure; eGFR, estimated glomerular filtration rate.

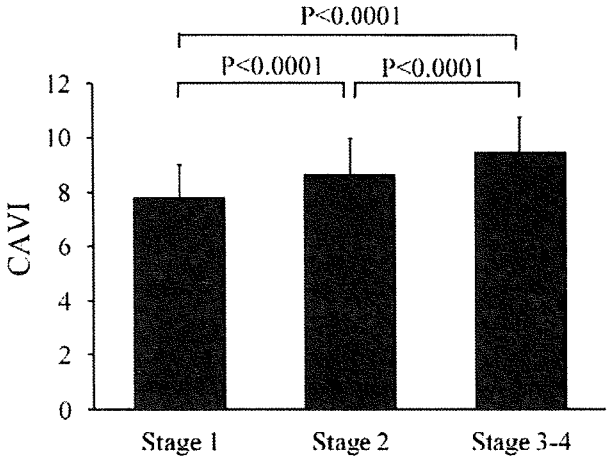

Fig. 2. Mean value of CAVI at different stages of CKD in the general population.

CAVI, cardio-ankle vascular index; CKD; chronic kidney disease.

(**Table 3**).

Comparison of CAVI in Stage of CKD

We analyzed the correlation between CAVI and CKD stages and showed that stepwise increments in CAVI occurred with deterioration in CKD from stage 1 to 4 (stage 1: 7.8 ± 1.2, stage 2: 8.7 ± 1.3, stage 3-4: 9.5 ± 1.3) (**Fig. 2**).

Relationship between CAVI and Proteinuria

We collected urinalysis data on 872 subjects and investigated the relationship between the prevalence of proteinuria and CAVI. Proteinuria was absent in 842 subjects and present in 30 subjects. CAVI in subjects without proteinuria was significantly higher than that measured in subjects with proteinuria (**Fig. 3**).

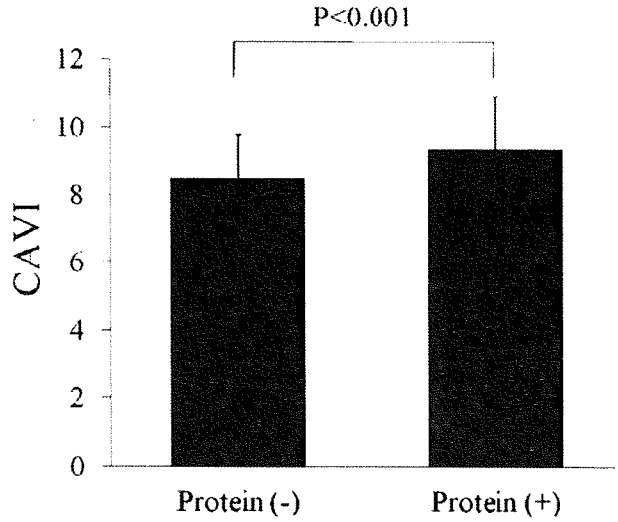

Fig. 3. Relationship between CAVI and proteinuria.

CAVI, cardio-ankle vascular index.

Discussion

In this study, we analyzed the association between CAVI and eGFR in the general Japanese population. Linear regression analysis demonstrated a significant correlation between CAVI and eGFR. Furthermore, multiple regression analysis using CAVI as an objective variable, adjusted for conventional atherosclerotic risk factors and eGFR as explanatory variables, revealed that CAVI was independently correlated with eGFR. In addition, there was a stepwise increase in CAVI that corresponded to increasing severity in CKD from stage 1 to 4.

A lower eGFR is associated with greater arterial stiffness and enhanced urinary albumin excretion, even at levels below those classified as microalbuminuria[14]. Measurement of baPWV has been used as a noninvasive clinical index of arterial stiffness[15], and has also been shown to predict the presence of coronary artery disease[16], and correlate with abdominal aortic calcification[17] and carotid intima-media thickness[18]. We have reported previously that baPWV correlates with age in healthy subjects, a finding suggesting that the index reflects age-related changes in vascular stiffness[19]. It has also been reported that eGFR is associated significantly with baPWV in Japanese patients, independent of traditional risk factors for cardiovascular disease[20]. In addition, Kawamoto *et al.* reported that PWV increased progressively with decreases in eGFR in community residents[11].

Several reports have shown the usefulness of

CAVI for the detection of atherosclerotic diseases[10, 21]. Ichihara *et al.* reported that CAVI reflects histological arterial fibrosis in hemodialysis patients and is a useful clinical marker for evaluating arterial stiffness in patients with kidney failure treated by hemodialysis[22]; however, there are no reports on the correlation between CAVI and eGFR in community residents and, to the best of our knowledge, this is the first report demonstrating that CAVI negatively correlates independently with eGFR in the general population.

The reason why CAVI showed a negative relationship with GFR warrants discussion. Renal changes may directly affect the viscoelastic arterial properties of arteries[23]. In addition, if a common factor interacts between large muscular-elastic arteries and the kidney, it seems likely that the extracellular matrix of vascular and renal cells is primarily involved[24]. These changes may obviously affect several pathways that control sodium and water balance[25], the renin-angiotensin-aldosterone system[26, 27], calcium-phosphate metabolism[28], and even vasoactive factors, such as nitric oxide, endothelin, and other compounds of endothelial origin[29, 30].

There are several limitations when measuring CAVI as it cannot be measured accurately in patients with aortic stenosis, peripheral arterial disease or atrial fibrillation. An ankle-brachial pressure index (ABI) <0.95 has been reported to be the cut-off value for diminished accuracy of baPWV measurements[31], and accordingly, CAVI cannot be measured accurately if the ABI is less than 0.95. In addition, there is no evidence that CAVI predicts mortality and morbidity in cardiovascular diseases. At present, this is also one of the limitations when assessing CAVI. Further studies are needed to evaluate the clinical value of CAVI.

In conclusion, we showed that the new arterial stiffness index, CAVI, negatively correlated with eGFR independently, indicating that CKD in the general Japanese population is associated with increased arterial stiffness.

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ORIGINAL ARTICLE

Noninvasive indices of arterial stiffness in hemodialysis patients

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The purpose of this study was to evaluate the validity of brachial–ankle pulse wave velocity (baPWV) and the cardio–ankle vascular index (CAVI) as measures of arterial stiffness in hemodialysis (HD) patients. We studied 160 consecutively enrolled HD patients (mean age: 59 ± 13 years; 91 male patients). We measured baPWV and CAVI using a VaSera VS-1000, maximum intima-media thickness (max IMT) of the carotid artery by ultrasonography and blood renal and lipid parameters. As a control, baPWV and CAVI were also measured in age- and gender-matched healthy volunteers. Both baPWV and CAVI were significantly higher in HD patients than in controls (baPWV: 1698 ± 355 vs. 1454 ± 263 cm s^{-1}, $P<0.0001$; CAVI: 9.3 ± 1.4 vs. 8.9 ± 1.2, $P<0.01$). BaPWV correlated positively with age ($r=0.549$, $P<0.0001$), systolic blood pressure (SBP) ($r=0.510$, $P<0.0001$), diastolic blood pressure ($r=0.203$, $P<0.0001$), pulse pressure (PP) ($r=0.499$, $P<0.0001$), KtV^{-1} ($r=0.221$, $P<0.01$), Brinkman index ($r=0.186$, $P<0.05$) and max IMT ($r=0.285$, $P<0.001$). CAVI also correlated positively with age ($r=0.562$, $P<0.0001$), SBP ($r=0.395$, $P<0.0001$), PP ($r=0.490$, $P<0.0001$), KtV^{-1} ($r=0.216$, $P<0.01$), Brinkman index ($r=0.238$, $P<0.01$) and max IMT ($r=0.280$, $P<0.001$). Multiple regression analysis demonstrated baPWV and CAVI correlated independently with age and SBP. Receiver operating characteristics (ROC) curve analysis demonstrated that baPWV and CAVI had similar power to predict increases in max IMT. We also measured baPWV and CAVI immediately before and after HD, and showed CAVI was influenced by changes in water volume. Both baPWV and CAVI are therefore useful indices of arterial stiffness in HD patients.
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Keywords: arterial stiffness; baPWV; CAVI; hemodialysis

INTRODUCTION

Chronic kidney disease is a worldwide public health problem and is a risk factor for the development of cardiovascular disease.[1] The incidence and associated costs of treatment of end-stage renal disease are increasing in Japan similar to that occurring in Western countries.[2,3] Accelerated atherosclerosis in end-stage renal disease patients is a serious problem and the resulting poor prognosis in hemodialysis (HD) patients is an important issue.[4,5]

Brachial–ankle pulse wave velocity (baPWV) is used as a noninvasive clinical index of arterial stiffness[6,7] and has been shown to predict the presence of coronary artery disease[8] and also correlate with abdominal aortic calcification[9] and carotid intima-media thickness.[10] Although baPWV is a useful index for measuring arterial stiffness in HD patients,[11–14] it has the limitation of being influenced by changes in blood pressure (BP) during measurements.

Recently, an atherosclerotic index, the cardio-ankle vascular index (CAVI), has been developed that involves measuring pulse wave velocity (PWV) and BP. CAVI is adjusted for BP based on the stiffness parameter β and therefore measures arterial stiffness independent of BP.[15–17] We have reported previously that CAVI showed a weaker correlation with systolic BP (SBP) than baPWV and was not affected by changes in BP during measurement.[9] There is also evidence that CAVI reflects histological arterial fibrosis and is a useful clinical marker for evaluating arterial stiffness in HD patients.[18] The purpose of the study was therefore to evaluate the validity of the noninvasive indices of arterial stiffness, baPWV and CAVI, in HD patients.

METHODS

Study subjects

We analyzed 160 consecutively enrolled patients with kidney failure who received HD therapy at Ueyama Hospital. The causes of kidney failure were nephritis in 120 patients, diabetes in 32 patients, collagen disease in seven patients and Fabry disease in one patient. Of these patients, 42 were treated with an angiotensin II receptor blocker (ARB), four with an angiotensin-converting enzyme inhibitor and 14 with a HMG-CoA reductase inhibitor. Exclusion criteria were diseases that affect baPWV and CAVI measurements including the presence of atrial fibrillation, a high frequency of ventricular and atrial premature beats, peripheral arterial disease with an ankle–brachial

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pressure index (ABI) of less than 0.9, heart failure (New York Heart Association class II and higher), pulmonary edema and cancer. To compare baPWV and CAVI between the HD patients and control subjects, 160 healthy volunteers matched with the HD patients for age and gender were enrolled in the study.

The protocol of this study was approved by the Institutional Review Board of Kagoshima University. Informed consent was given by all the patients and volunteers.

Measurements of baPWV and CAVI

Immediately before HD, baPWV and CAVI were measured using a Vasera VS-1000 (Fukuda Denshi, Tokyo, Japan). As reported previously, cuffs were placed on both ankles and the brachium, which were not used for blood access. Electrocardiographic electrodes were attached to the upper arm and a microphone was placed on the sternal angle for phonocardiography. The subjects rested in the supine position for 5 min. PWV was calculated by dividing the distance from the aortic valve to the ankle artery by the sum of the difference between the time the pulse waves were transmitted to the brachium and the time the same waves were transmitted to the ankle, and the time difference between the second heart sound on the phonocardiogram and the notch of the brachial pulse waves.[15–17] To minimize cuff inflation effects on blood flow dynamics, pulse waves were measured with the cuffs inflated to lower than diastolic pressure (50 mm Hg). The extremity blood pressure was then measured by oscillometry. SBP, diastolic BP (DBP) and pulse pressure (PP) were obtained by measuring the BP at the right brachial artery.

CAVI was calculated by the following equation: $CAVI=a[\{2\rho\times 1/(SBP-DBP)\}\times\{\ln(SBP/DBP)\times PWV^2\}]+b$ (ρ: density of blood, a and b: constants).[16,17,19]

Analysis of blood samples

Blood samples were obtained after an overnight fast on the morning of the day baPWV and CAVI were measured. The serum concentrations of creatinine (Cr), blood urea nitrogen (BUN), uric acid (UA), calcium (Ca), phosphoric acid (P), parathyroid hormone (PTH), total cholesterol, triglyceride (TG) and high-density lipoprotein cholesterol (HDL-C) were measured using standard laboratory procedures. Low-density lipoprotein cholesterol (LDL-C) was calculated by the Friedewald equation. $Kt\,V^{-1}$ was determined according to the procedure of Shinzato *et al.*[20]

Measurement of maximum IMT

The maximum IMT (max IMT) of the carotid artery was evaluated by high-resolution ultrasonography (Fukuda Denshi) using a 7.5-MHz probe. The IMT was measured as the distance from the leading edge of the first echogenic line to the leading edge of the second echogenic line. In this study, the thickest point of the bilateral carotid artery was defined as the max IMT without plaque.

Statistical analysis

Data are expressed as the mean ± s.d. Differences between the mean values of the two groups were analyzed by unpaired *t*-tests, whereas differences between mean values of measurements at two points were analyzed using paired *t*-tests. The relationship between continuous variables was analyzed by linear regression analysis and independent associations between variables were examined by multiple regression analysis. The statistical analyses were performed with Statview version 5.0 (SAS Institute, Cary, NC, USA), whereas receiver operating characteristics (ROC) curve analysis was performed with JMP version 5.1.1 (SAS Institute). *P*-values less than 0.05 were considered statistically significant.

RESULTS

Comparison of baPWV and CAVI between HD patients and controls

The clinical characteristics of the HD patients and age- and gender-matched control subjects are summarized and compared in Table 1.

Table 1 Clinical characteristics of the control and HD groups

Variable	*Control (n=160)*	*HD (n=160)*	P-*value*
Age (years)	60 ± 10	60 ± 13	NS
Gender (M/F)	91/69	91/69	NS
BMI ($kg\,m^{-2}$)	23.2 ± 2.8	21.6 ± 7.1	<0.05
SBP (mm Hg)	127 ± 14	148 ± 22	<0.0001
DBP (mm Hg)	83 ± 10	87 ± 13	<0.001
PP	45 ± 9	62 ± 17	<0.001
Cr (mg per 100 ml)	0.8 ± 0.2	11.0 ± 3.0	<0.0001
BUN (mg per 100 ml)	16 ± 4	71 ± 16	<0.0001
UA (mg per 100 ml)	5.2 ± 1.3	6.8 ± 1.4	<0.0001
HDL-C (mg per 100 ml)	57 ± 14	54 ± 17	NS
LDL-C (mg per 100 ml)	130 ± 31	80 ± 27	<0.0001
TG (mg per 100 ml)	112 ± 69	104 ± 62	NS
Duration of dialysis (days)	—	3138 ± 2498	ND
Medication			
ARB	0	42	ND
ACE-I	0	4	ND
Statin	0	14	ND

Abbreviations: ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; BMI, body mass index; BUN, blood urea nitrogen; Cr, serum creatinine; DBP, diastolic blood pressure; HD, hemodialysis; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ND, not done; NS, not significant; PP, pulse pressure; SBP, systolic blood pressure; statin, HMG-CoA reductase inhibitor; TG, triglyceride; UA, uric acid.

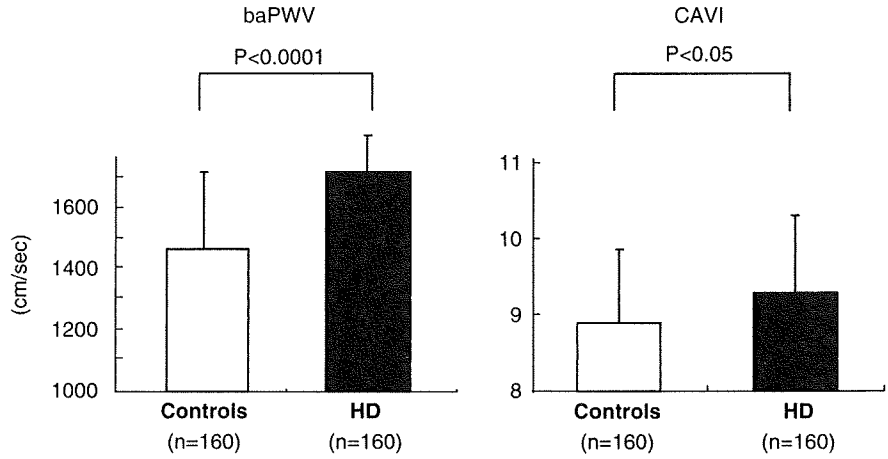

Figure 1 Comparison of baPWV and CAVI between HD patients and controls. baPWV, brachial–ankle pulse wave velocity; CAVI, cardio–ankle vascular index; HD, hemodialysis.

There was no significant difference in age, gender, HDL-C and TG between the two groups, whereas SBP, DBP and PP were significantly higher and BMI and LDL-C significantly lower in the HD patients compared with the control group. In addition, both baPWV and CAVI were significantly higher in the HD patients than in the control group (Figure 1).

Table 2 Correlation coefficients between baPWV or CAVI and other variables in HD patients calculated by linear regression analysis

	baPWV		*CAVI*	
Variables	R	*P-value*	R	*P-value*
Age (years)	0.549	<0.0001	0.562	<0.0001
SBP (mm Hg)	0.510	<0.0001	0.395	<0.0001
DBP (mm Hg)	0.203	<0.0001	0.018	NS
PP	0.499	<0.0001	0.490	<0.0001
Max IMT	0.285	<0.001	0.280	<0.001
KtV^{-1}	0.221	<0.01	0.216	<0.01
Duration of dialysis	0.375	<0.0001	0.390	<0.0001
Brinkman index	0.186	<0.05	0.238	<0.01
BMI	0.020	NS	0.041	NS
LDL-C	0.051	NS	0.064	NS
HDL-C	0.155	NS	0.131	NS
TG	0.067	NS	0.001	NS
ip×Ca	0.101	NS	0.082	NS
i PTH	0.104	NS	0.126	NS

Abbreviations: baPWV, brachial–ankle pulse wave velocity; BMI, body mass index; CAVI, cardio–ankle vascular index; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; i PTH, intact parathyroid hormone; LDL-C, low-density lipoprotein cholesterol; Max IMT, maximum intima-media thickness; NS, not significant; PP, pulse pressure; SBP, systolic blood pressure; TG, triglyceride.

Linear regression analysis of baPWV or CAVI and other variables in HD patients

The results of the linear regression analysis of baPWV and the other variables in the HD patients are shown in Table 2. Positive correlations were found between baPWV and age, SBP, DBP, PP, KtV^{-1}, Brinkman index and max IMT and between CAVI and age, SBP, PP, KtV^{-1}, Brinkman index and max IMT. Although baPWV and CAVI showed a similar degree of correlation with age, CAVI was more weakly correlated with SBP than baPWV (Figure 2).

Multiple regression analysis between baPWV or CAVI and other variables in HD patients

Table 3 shows the results of a multiple regression analysis with baPWV or CAVI as the dependent variable, and age, SBP, PP, max IMT, KtV^{-1}, duration of dialysis and diabetes mellitus as the independent variables. In HD patients, baPWV correlated independently with age, SBP and PP, whereas CAVI correlated independently with age, SBP, KtV^{-1} and diabetes mellitus.

ROC curve analysis between baPWV or CAVI and increased max IMT ($\geqslant$0.9 mm)

Figure 3 demonstrates the ROC curves of baPWV and CAVI to predict increased max IMT ($\geqslant$0.9 mm). The area under the ROC curve (AUC) for baPWV was 0.66, with the highest discriminating sensitivity and specificity being 0.76 and 0.59, respectively at baPWV=1563 cm s^{-1}. In contrast, the AUC for CAVI was 0.65 and the highest discriminating sensitivity and specificity were 0.57 and 0.70, respectively at CAVI=9.5. These results suggest that baPWV and CAVI have similar power to predict the increases in max IMT.

Effect of water volume changes on baPWV and CAVI

To analyze the effect of HD on baPWV and CAVI, we measured both indices immediately before and after HD in 101 patients (Table 4). In these patients, SBP, DBP and baPWV decreased significantly after HD,

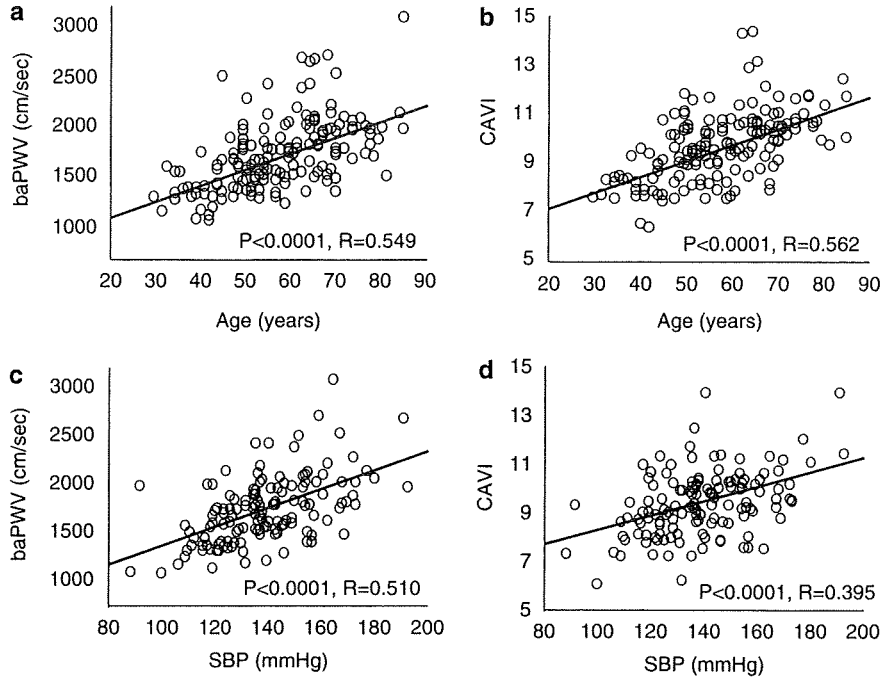

Figure 2 Relationship between baPWV and age (**a**), CAVI and age (**b**), baPWV and SBP (**c**) and CAVI and SBP (**d**). baPWV, brachial–ankle pulse wave velocity; CAVI, cardio–ankle vascular index; SBP, systolic blood pressure.

whereas CAVI increased significantly after HD. We also determined the water removal rate as follows; (body weight before HD−body weight after HD)/dry weight. CAVI increased significantly after HD in patients with a water removal rate >5%, but did not change in patients with a water removal rate ⩽5%. In contrast, baPWV decreased significantly after HD with no influence of water removal rate being observed. These results suggest that CAVI is influenced by changes in water volume.

Table 3 Multiple regression analysis between baPWV or CAVI and other variables in HD patients

	baPWV		*CAVI*	
Variable	*Coefficient*	*P-value*	*Coefficient*	*P-value*
Age (years)	13.987	<0.0001	0.056	<0.0001
SBP (mm Hg)	9.689	<0.0001	0.017	<0.05
PP	−8.078	<0.05		NS
Max IMT		NS		NS
KtV^{-1}		NS	−0.789	<0.05
Duration of dialysis		NS		NS
HDL-C		NS		NS
DM		NS	0.73	<0.05

Abbreviations: baPWV, brachial–ankle pulse wave velocity; CAVI, cardio–ankle vascular index; Cr, serum creatinine; DM, diabetic mellitus; HDL-C, high density lipoprotein cholesterol; Max IMT, maximum intima-media thickness; PP, pulse pressure; SBP, systolic blood pressure.

DISCUSSION

This study demonstrated that baPWV and CAVI were both significantly higher in HD patients than in age- and gender-matched controls. In HD patients, baPWV and CAVI showed positive correlations with age, SBP, PP, KtV^{-1}, Brinkman index and max IMT. Multiple regression analysis revealed that baPWV and CAVI were correlated independently with age and SBP. ROC curve analyses demonstrated that baPWV and CAVI had similar power to predict increases in max IMT. Furthermore, we measured baPWV and CAVI immediately before and after HD and showed CAVI was influenced by changes in water volume.

The baPWV is a noninvasive clinical index of arterial stiffness in HD patients. It has been reported that glycated albumin, but not glycated hemoglobin or plasma glucose, is independently and positively associated with baPWV in HD patients with type 2 diabetes.[12] Kobayashi *et al.*[21] also reported a positive association between blood rheology and carotid IMT and baPWV in 118 HD patients. Furthermore, there is evidence to show that baPWV is useful for identifying a high-risk population of HD patients with an ABI greater than 0.9.[11] In addition, it has been reported that baPWV measurement shows variations at different time points in HD patients.[22] Su *et al.*[22] measured baPWV before and after HD and then on the next dialysis-free day and found baPWV increased significantly after HD, despite a significant decrease in body weight and BP.

CAVI is a useful index of arterial distensibility[16] and is not influenced by BP changes during measurement.[17] Several studies have shown the usefulness of CAVI for detecting atherosclerotic disease,[23,24] with one study in 67 HD patients using multiple regression analysis to reveal that the high-molecular weight adiponectin to

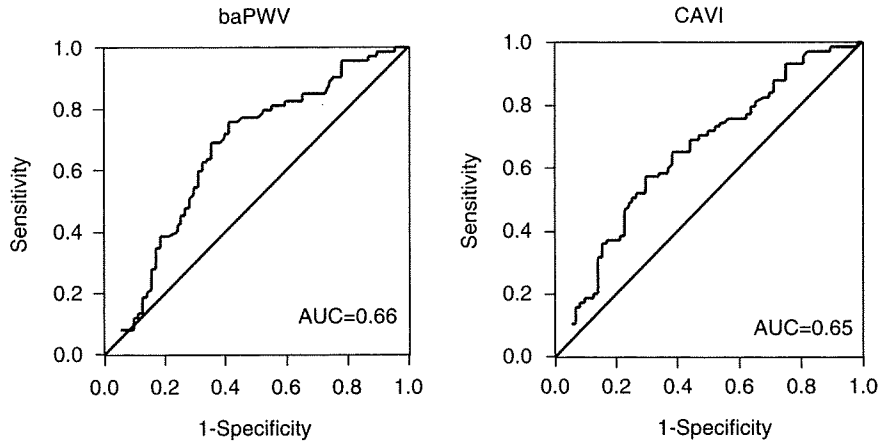

Figure 3 Receiver operating characteristic (ROC) curves of the ability of baPWV and CAVI to predict increases in IMT (⩾0.9 mm). AUC, area under ROC curve; baPWV, brachial–ankle pulse wave velocity; CAVI, cardio–ankle vascular index.

Table 4 Effect of changes in water volume on baPWV and CAVI

	Total (n=101)			*Water removal rate ⩽5% (n=33)*			*Water removal rate >5% (n=68)*		
	Before HD	*After HD*	*P-value*	*Before HD*	*After HD*	*P-value*	*Before HD*	*After HD*	*P-value*
Body weight (kg)	53.6±9.9	50.8±9.6	<0.0001	55.7±9.2	53.6±8.9	<0.0001	52.7±10.1	49.4±9.6	<0.0001
SBP (mm Hg)	155±23	125±20	<0.0001	144±21	125±22	<0.0001	159±23	125±20	<0.0001
DBP (mm Hg)	86±12	76±12	<0.0001	81±10	73±11	<0.001	89±11	78±12	<0.0001
baPWV ($cm\,s^{-1}$)	1794±394	1711±385	<0.05	1740±334	1656±320	<0.05	1820±419	1737±412	<0.05
CAVI	8.9±1.4	9.4±1.4	<0.0001	9.3±1.4	9.4±1.4	NS	8.7±1.3	9.4±1.4	<0.0001

Abbreviations: baPWV, brachial–ankle pulse wave velocity; CAVI, cardio–ankle vascular index; DBP, diastolic blood pressure; HD, hemodialysis; SBP, systolic blood pressure.

total adiponectin ratio was an independent determinant of PWV and CAVI.[25] Takaki *et al.*[26] compared baPWV and CAVI in 130 patients with chest pain syndrome and concluded that CAVI was superior to baPWV as a parameter of arterial stiffness. A further study in 103 HD patients used multiple regression analysis to show a significant association between histological arterial fibrosis and CAVI, but no such relationship with PWV.[18] Ichihara *et al.*[13,14] reported that several drugs including an angiotensin II receptor blocker (losartan), angiotensin-converting enzyme-I (tradolapril) and HMG-CoA reductase inhibitor (fluvastatin) improved arterial stiffness measured by PWV. However, in our study, there was no significant difference in baPWV or CAVI in patients either taking or not taking an angiotensin II receptor blocker, angiotensin-converting enzyme-I or HMG-CoA reductase inhibitor (data not shown). The previous studies by Ichihara *et al.*[13,14] were longitudinal studies, whereas the present study was a cross-sectional design.

We demonstrated that CAVI increased significantly after HD in patients with a removal rate >5%. Large volume reduction after HD may increase the sympathetic nervous activity, which increases the vascular tone. The increase of vascular tone may result in the increase of CAVI. In addition, the hemoconcentration after HD may increase the CAVI, because the density of blood (ρ) is one of the determinants of CAVI.

There are several limitations in the measurement of CAVI as it cannot be determined accurately in patients with aortic stenosis, peripheral arterial disease or atrial fibrillation. An ABI <0.95 has been reported to be the cutoff value for diminished baPWV accuracy,[27] and therefore CAVI cannot be measured accurately if the ABI is less than 0.95. Further prospective studies are therefore needed to evaluate the validity of CAVI in the clinical assessment and prediction of mortality in HD patients.

In conclusion, baPWV and CAVI in HD patients were significantly higher than in age- and gender-matched control subjects, with both indices correlating independently with age and SBP in the HD patients. ROC curve analysis demonstrated that the power of baPWV and CAVI to predict increases in max IMT was similar. Furthermore, we measured baPWV and CAVI immediately before and after HD, and showed CAVI was influenced by changes in water volume. Therefore, both baPWV and CAVI are useful indices of arterial stiffness in HD patients.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Tacrolimus-eluting stent inhibits neointimal hyperplasia via calcineurin/NFAT signaling in porcine coronary artery model

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ABSTRACT

Aims: The purpose is to elucidate the mechanism by which a newly developed tacrolimus-eluting stent (TES) prevents neointimal hyperplasia after stenting.

Methods and results: The three major coronary arteries in juvenile swine were randomized to implantation of either a TES or bare metal stent (BMS). Twelve weeks after stenting, the TES showed 29% less neointimal area than the BMS. Immunohistochemical staining showed that the expression of calcineurin was up-regulated in the neointima and media after stenting, and the TES inhibited this up-regulation. Western blotting demonstrated that the expression of calcineurin, nuclear factor of activated T cell (NFAT), and interleukin-2 (IL-2) was lower with the TES than with the BMS. To confirm the effect of tacrolimus on vascular smooth muscle cells (VSMCs) and its mechanism, cultured rat VSMCs were incubated with 12.5 μM of tacrolimus (tacrolimus group) or without tacrolimus (control group). The cell number of the tacrolimus group was significantly lower than that of the control group at 48 h of incubation. Western blotting demonstrated that tacrolimus decreased the expression of calcineurin, NFATc4, and IL-2 of cultured VSMCs. We confirmed that calcineurin small-interfering RNA (siRNA) decreased cell proliferation and the expression of NFATc4 and IL-2 in cultured VSMCs compared with negative control-siRNA.

Conclusion: The newly developed TES inhibited neointimal hyperplasia after stenting via the calcineurin/NFAT/IL-2 signaling pathway, which is one of several mechanisms through which TES inhibits restenosis. Calcineurin may be an important molecular target to prevent restenosis after stenting.

1. Introduction

Percutaneous coronary intervention (PCI) is a useful procedure for the treatment of coronary stenosis. Restenosis has been called the "Achilles heel" of coronary stenting and is caused by a combination of factors, including neointimal proliferation, elastic recoil, reorganization of thrombus, remodeling, and inflammation [1,2]. Currently, a number of drug-eluting stents (DES) have been developed, including different carrier stents, coatings and drugs; and these new DES are under evaluation for their effectiveness and safety [3,4] and [5]. Sirolimus-eluting stents (SESs) and paclitaxel-eluting stents (PESs) strongly reduced the incidence of restenosis compared with bare metal stents (BMSs) [6,7]. Recently, many kinds of drug-eluting stents have been investigated in the clinical setting. Positive clinical data on drug-eluting stents come from trials examining SESs in the SIRIUS Trial [8] and PESs in TAXUS-series Trial [9]. A pooled analysis demonstrated that both SESs and PESs were associated with a marked reduction in target-lesion revascularization. However, stent thrombosis after 1-year was more common with both SESs and PESs than with BMSs [10]. A systematic autopsy study reported that both SESs and PESs caused significant delay in arterial healing characterized by persistent fibrin deposition and delayed re-endothelialization when compared with sites of BMS implantation [11,12].

To reduce the risk of in-stent restenosis and interference with the natural healing response, Kaneka Corporation (Osaka, Japan) has developed a new drug-eluting stent. The new stent is a combination of a cobalt chrome (CoCr) stent, a biodegradable polymer and a pharmaceutical agent, tacrolimus [13]. Tacrolimus is a water-insoluble macrolide cytostatic immunosuppressant with both anti-proliferative and anti-inflammatory activity [14]. It has been used clinically to prevent renal and liver transplant rejection. Tacrolimus binds to the intracellular FK-binding protein (FKBP) and forms a complex that binds to calcineurin. This binding inhibits the activation of calcineurin and disrupts the dephosphorylation of nuclear factor of activated T cell (NFAT) in T cells [15]. In addition, cell culture experiments indicated that tacrolimus inhibited the proliferation of vascular smooth muscle cells (VSMCs) [16]. However, the mechanism of the anti-proliferative effect of tacrolimus on VSMCs has not yet been clarified.

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The purpose of this study is to elucidate the precise mechanism by which the newly developed tacrolimus-eluting stent (TES) prevents hyperplasia after stenting, using a porcine coronary model and cultured human VSMCs.

2. Materials and methods

2.1. Stent characteristics

The stent platform of this product is the balloon-expandable stent developed by the Kaneka Corporation and is made of CoCr alloy. CoCr has been successfully used for human implants such as artificial joints and dental implants. The stent is made by cutting an alloy tube with a laser and consists of two helical coils intercrossed with two phase-different links on each turn. The stent strength in the radial direction, recoil ratio, and stent shortening (the percentage change in length between the mounted condition and the expanded condition) is a medium level compared with other stents (Additional Fig. 1A). The whole surface of the stent platform is coated with a layer of a biodegradable polymer and tacrolimus. As shown in Additional Fig. 1B and C, the surface is generally smooth and there are no cracks or peelings caused by expansion. A cross-section on transmission electron microscopy shows that the drug is uniformly distributed in the polymer layer (Additional Fig. 1D).

2.2. Animal model

All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication no. 85-23, revised 1996). The protocol for the animal research was approved by a local ethics review board.

Female juvenile swine LWD with a body weight of 40–60 kg were used. Swine were pre-sedated with intramuscular injection of ketamine (5 mg/kg) and xylazine (2 mg/kg). Anesthesia was initiated by 5% isoflurane, followed by orotracheal intubation and ventilation with 1.0–3.0% isoflurane, 70% N_2O, and 30% oxygen. After surgical exposure, the right femoral artery was punctured and a 6 Fr sheath was placed. The animals then received 200 IU/kg intravenous heparin. The coronary arteries were imaged using a standard angiographic technique. Target segments were selected in the right (RCA), the left anterior descending (LAD) and the left circumflex (LCX) coronary arteries. After stent implantation, cefazolin sodium (0.5 g/day) was administered by intramuscular injection for 3 days after the surgical procedure. Oral aspirin (330 mg/day) and ticlopidine (250 mg/day) were administered starting 1-day before the procedure and continuing until sacrifice. Stents (3.5 mm in diameter and 15 mm in length) were implanted in coronary arteries with a diameter of 2.8 mm (stent-to-vessel ratio of 1.25:1). Coronary vessels were randomized to receive a bare metal stent (BMS) or a TES (two TESs in two RCAs, two TESs in two LADs, two TESs in two LCxs, two BMSs in two RCAs, two BMSs in two LADs, two BMSs in two LCxs). There is no structural difference between the TES and the BMS, or differences in the delivery/deployment of the stent. The animals were euthanized and the stented coronary arteries were harvested at 2, 4, or 12 weeks after stent implantation for immunohistochemistry, at 12 weeks for morphometry, and at 4 weeks for Western blot analysis.

2.3. Quantitative coronary angiography (QCA)

Coronary imaging was performed using a GE Healthcare OEC 9800. An experienced investigator who was blinded to the randomization assignment measured the reference diameter and the minimal luminal diameter of the stented segments at follow-up angiography. The % diameter stenosis was calculated as follows: % diameter stenosis = (reference diameter − minimal luminal diameter)/reference diameter × 100.

2.4. Morphometric measurements

For the morphometric analysis, the heart was excised, and the coronary arteries were fixed with 10% buffered formalin and embedded in resin and cut into 5 μm-thick cross-sections. Each cross-section was stained by hematoxylin-eosin (HE). After digitalizing, histomorphometric measurements were performed with Scion Image (Scion Corporation, Frederick, MD) [17]. The vessel area and neointimal area in each proximal, middle and distal stented region were measured, and the % area stenosis was calculated as follows: % area stenosis = neointimal area/vessel area × 100. The % area stenoses in the three parts (proximal, middle and distal) were averaged. Researchers were blinded to the stent type.

2.5. Immunohistochemistry

For immunohistochemistry, the stent sites were dissected into blocks, and the stent wires were carefully removed. Immunohistochemical staining was carried out on paraffin-embedded sections as described previously [18]. After deparaffinization and hydration

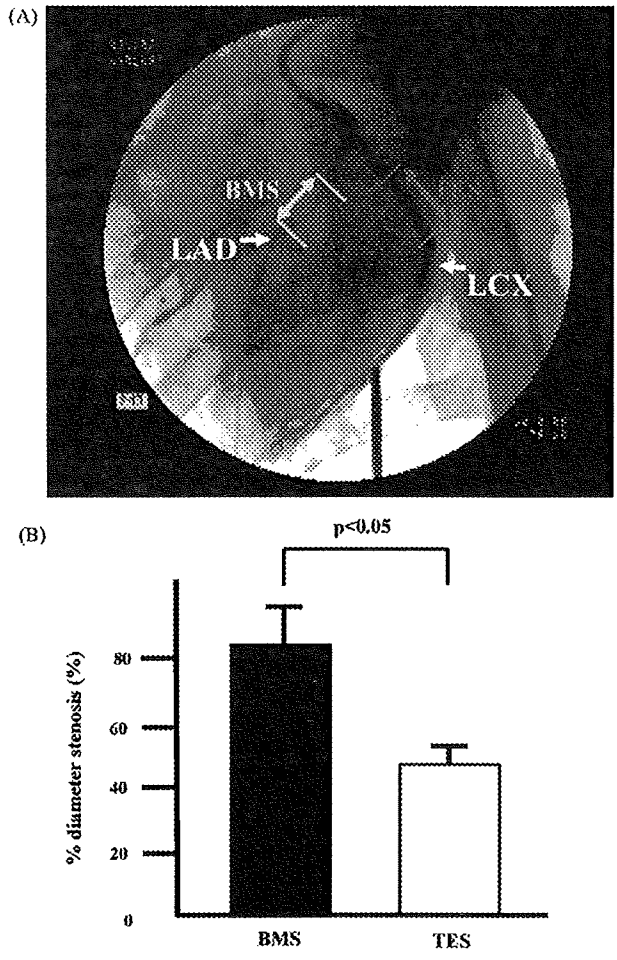

Fig. 1. Quantitative coronary angiography (QCA). (A) Representative coronary angiography 12 weeks after stent implantation shows implantation of a bare metal stent (BMS) in the left anterior descending coronary artery (LAD) and implantation of a tacrolimus-eluting stent (TES) in the left circumflex coronary artery (LCX). (B) The % diameter stenosis measured by QCA 4 weeks after stent implantation. The % diameter stenosis of the TES was significantly smaller than that of the BMS ($n = 6$).

of specimens, endogenous peroxidase activity was blocked and the specimens were fixed by immersion in 0.3% H_2O_2 in methanol for 20 min. Immunohistochemical staining was performed with a goat polyclonal antibody against human calcineurin; PP2B-Aα, von Willebrand factor (vWF), and endothelial nitric oxide synthase (eNOS) (Santa Cruz Biotechnology, Santa Cruz, CA), by the use of the labeled streptavidin biotin complex method (Simple-stain MAX-PO kit, Nichirei, Tokyo, Japan). After blocking with 10% rabbit or goat serum, slides were incubated overnight with a primary antibody at 4 °C in a moisture chamber. Slides were washed with Tris-buffered saline (TBS) and incubated with a biotinylated secondary antibody at room temperature for 30 min. After washing with TBS, slides were incubated with streptavidin at room temperature for 30 min and visualized with 3,3′-diaminobenzidine.

2.6. Western blot analysis

Protein was extracted from the neointima and media of porcine coronary arteries using the Protein and RNA Isolation System (Ambion Inc., Austin, TX). Insoluble matter was removed by centrifugation, and the protein concentration was measured by a bicinchoninic acid assay (PIERCE Biotechnology Inc., Rockford, IL). Western blotting was performed with a NuPAGE™ Electrophoresis System (Invitrogen, Carlsbad, CA) as reported previously [19]. Briefly, 10-μg protein samples were resuspended in reduced sample buffer, and then electrophoresed on a 4–12% Bis–Tris gel (Invitrogen, Carlsbad, CA) with MOPS running buffer, blotted to a nitrocellulose membrane. The protein sample was then sequentially probed with a goat polyclonal antibody against human calcineurin (PP2B-Aα), a rabbit polyclonal antibody against human NFATc4 (Santa Cruz Biotechnology), a rabbit polyclonal antibody against human interleukin-2 (IL-2) (Santa Cruz Biotechnology), and a rabbit polyclonal antibody against human actin (Santa Cruz Biotechnology). Horseradish peroxidase-conjugated rabbit anti-goat antibody (Santa Cruz Biotechnology) or donkey anti-rabbit antibody (Santa Cruz Biotechnology) were then added, and the secondary antibody was detected by autoradiography using enhanced chemiluminescence (ECL Plus, GE Healthcare UK Ltd., Little Chalfont, UK). Densitometric analysis was performed to quantitate calcineurin, NFATc4, IL-2, and actin protein using NIH image software. Actin protein was used as a reference for quantitation of calcineurin, NFATc4, and IL-2 protein.

2.7. Cell experiments

VSMCs were isolated from the media of rat aorta. Cell culture experiments were performed as reported previously [20]. Rat VSMCs (5000) cells were seeded into 48 wells (AGC Techno Glass Co., Ltd., Chiba, Japan) in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Carlsbad, CA) supplemented with 1% fetal

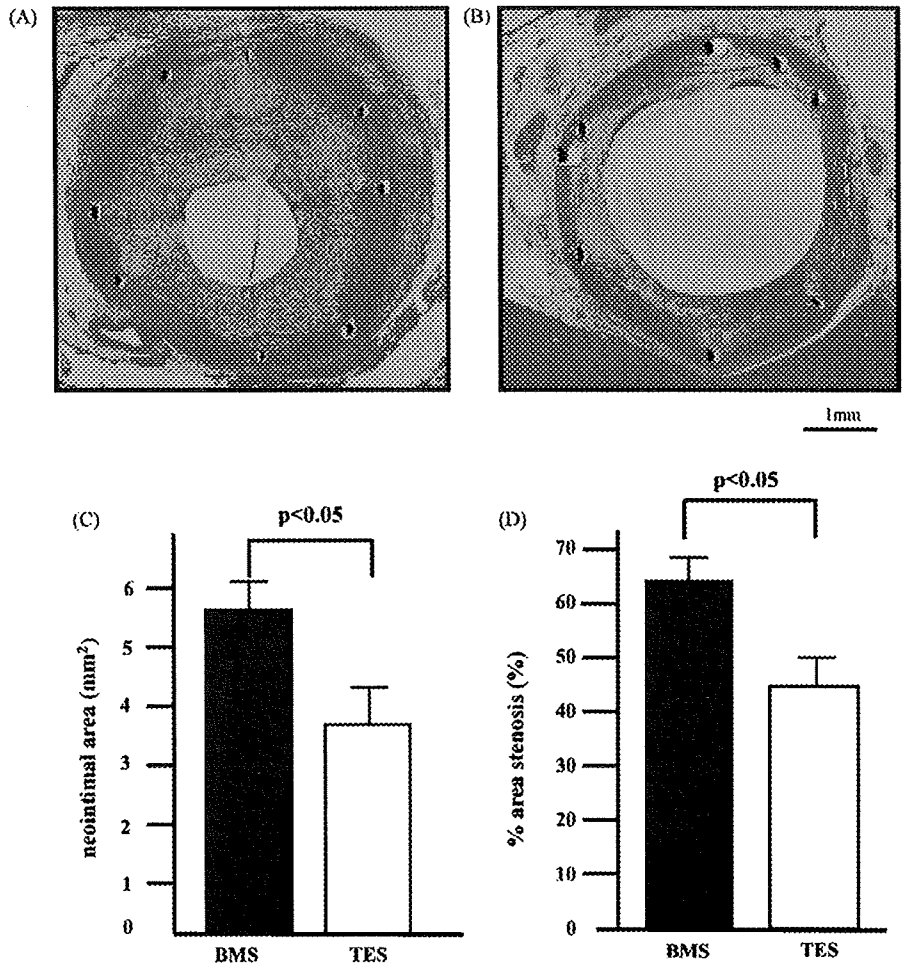

Fig. 2. Morphometric analysis at 12 weeks after stent implantation. (A and B) HE staining of cross-sections of coronary arteries implanted with a bare metal stent (BMS) (A) and a tacrolimus-eluting stent (TES) (B). (C) The neointimal area was significantly smaller with a TES than with a BMS ($n=6$). (D) The % area stenosis measured by histomorphometry was significantly smaller in TES compared with BMS ($n=6$).

bovine serum (FBS; SAFC Biosciences, Wicklow, Ireland), and 100 U/mL of penicillin and gentamicin. VSMCs used for experiments were from the fifth to the ninth passages. After 24 h, we exchanged the DMEM containing 1% FBS with or without the addition of 12.5 μM of tacrolimus. The cells without tacrolimus served as a control group. At 48 h of incubation, the cells were harvested by tripsinization and counted in a CDA-500 Particle Analyzer (Sysmex Corporation, Hyogo, Japan).

For Western blotting, rat VSMCs were cultured in 25 cm^2 plates (Agc Techno Glass Co., Ltd., Chiba, Japan) with DMEM containing 1% FBS. After 24 h, we exchanged the DMEM containing 1% FBS with or without the addition of 12.5 μM of tacrolimus. The cells without tacrolimus served as the control group. At 48 h of incubation, cytoplasmic and nuclear proteins were extracted from cultured VSMCs using a Protein and RNA Isolation System. We examined the expression of calcineurin, NFATc4, and IL-2 as described in Section 2.6.

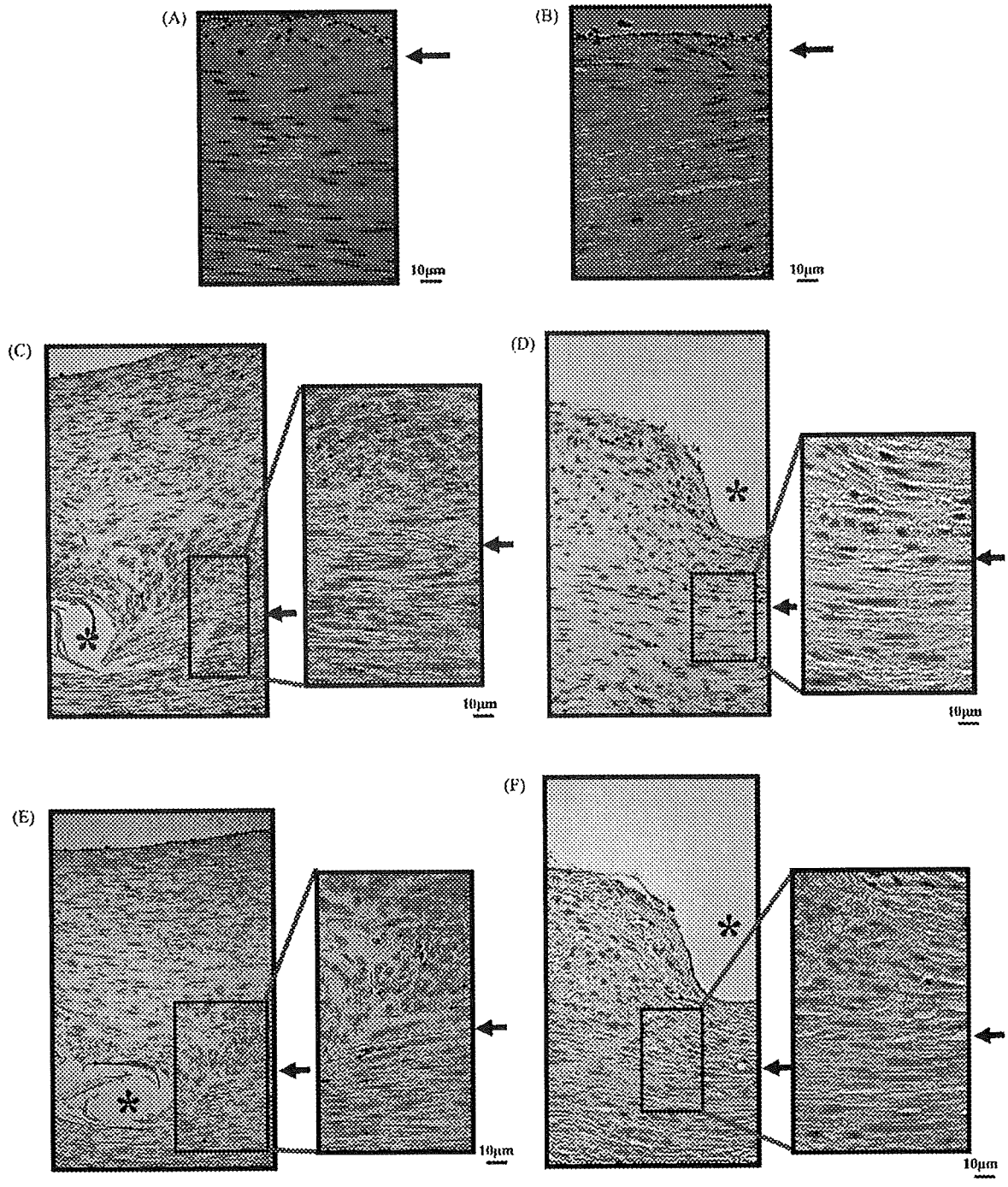

Fig. 3. Immunohistochemical staining for calcineurin in porcine coronary arteries after stenting. Calcineurin was up-regulated in the medial and neointimal smooth muscle cells (SMCs) at 2 (A), 4 (C), and 12 (E) weeks after stenting with a bare metal stent (BMS). In contrast, after stenting with a tacrolimus-eluting stent (TES), the expression of calcineurin was suppressed in the medial and neointimal SMCs at 2 (B), 4 (D) and 12 (F) weeks after stenting. Arrows demonstrate the internal elastic lamina, (*) the space of the stent. Bar, 10 μm.

2.8. Small-interfering RNA transfection

We used small-interfering RNA (siRNA) to suppress the expression of calcineurin. Calcineurin-siRNA (Cat #4390815) and negative control-siRNA (control-siRNA), a 21-nucleotide RNA duplex with no known sequence homology (Cat #4635), were purchased from Ambion (Austin, TX). For siRNA transfection, rat VSMCs (density, 4×10^3/well) were cultured on 48 well plates with medium containing 5% FBS. Transfection of siRNA into cells was achieved by the use of siPORT NeoFX transfection agent and Opti-MEM (Invitrogen) according to the manuals. We mixed the diluted siPORT NeoFX transfection agent and the diluted calcineurin-siRNA, dispensed into cultured wells, and added DMEM containing 5% FBS. 72 h after transfection, we analyzed cell number and isolated protein for Western blotting as described in Section 2.7.

2.9. Statistical analysis

All calculated data are presented as the mean ± SD. Statistical significance was evaluated using unpaired Student's *t*-test for comparisons between two groups. A probability value of $P < 0.05$ was considered to indicate statistical significance.

3. Results

3.1. Quantitative coronary angiography

The coronary arteries of juvenile swine were subjected to implantation of either a BMS or a TES (Fig. 1A). At 12 weeks after stenting, QCA demonstrated that % diameter stenosis of the TES was significantly smaller than that of the BMS (TES: 48.4 ± 13.6% versus BMS: 82.5 ± 16.2%, $P < 0.005$, $n = 6$ in each group) (Fig. 1B).

3.2. Morphometric measurement

Fig. 2A and B shows representative photographs of cross-sections of coronary arteries implanted with a BMS and TES and stained with HE. The histomorphometric measurement showed that the neointimal area was significantly smaller with the TES than with the BMS (TES: 3.87 ± 1.18 mm^2 versus BMS: 5.81 ± 0.70 mm^2, $P < 0.05$, $n = 6$ in each group, Fig. 2C). The % area stenosis measured by histomorphometry was significantly smaller in the TES than the BMS (TES: 45.3 ± 7.2% versus BMS: 64.4 ± 14.7%, $P < 0.05$, $n = 6$ in each group, Fig. 2D).

3.3. Immunohistochemical analysis

Fig. 3 shows the immunohistochemical staining of calcineurin in each group at 2, 4, and 12 weeks after stenting. Immunohistochemical staining demonstrated that calcineurin was not expressed in the medial VSMCs of non-injured coronary arteries (data not shown); it was up-regulated in the medial and neointimal VSMCs at 2, 4, and 12 weeks after stenting with a BMS. In contrast, in coronary arteries implanted with a TES, the protein expression of calcineurin was suppressed in the medial and neointimal VSMCs at 2, 4 and 12 weeks after stenting.

We compared the effect of TES and BMS on re-endothelialization and endothelial function 4 weeks after stenting. To investigate the degree of re-endothelialization, the percentage of vWF-positive length of luminal side was measured [21]. There was no significant difference in the percentage of vWF-positive length between TES and BMS (Additional Fig. 3A). In addition, we evaluated endothelial cell function using immunohistochemistry for eNOS. The expression of eNOS in the endothelial cells was similar with TES and BMS (Additional Fig. 3B).

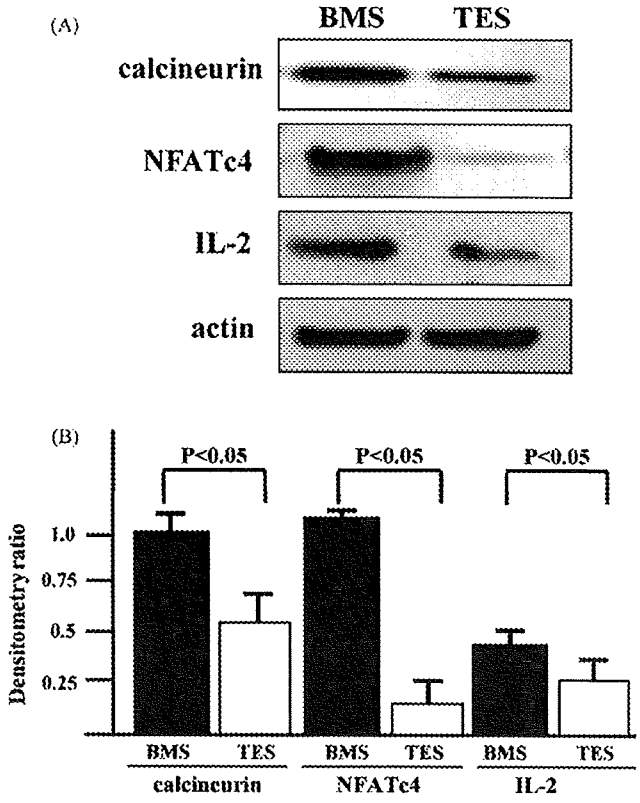

Fig. 4. Western blot analysis for calcineurin, nuclear factor of activated T cell (NFAT), interleukin-2 (IL-2), and actin in neointima of porcine coronary arteries at 4 weeks after implantation of a tacrolimus-eluting stent (TES) or a bare metal stent (BMS). (A) Representative Western blot analysis for calcineurin, NFATc4, IL-2, and actin in BMS and TES. (B) Densitometric analysis of Western blotting demonstrated that the expression of calcineurin, NFATc4, and IL-2 with the TES was significantly lower compared with the BMS.

3.4. Western blot analysis

We isolated protein from porcine coronary arteries at 4 weeks after stenting and used it for Western blotting to analyze the expression of calcineurin, NFATc4, and IL-2. Densitometric analysis of Western blotting demonstrated that the expression of calcineurin, NFATc4, and IL-2 with the TES was significantly lower compared with the BMS (Fig. 4). These results suggested that tacrolimus inhibited the expression of the calcineurin/NFAT/IL-2 pathway that was up-regulated by stenting.

3.5. Cell experiments

In order to confirm the effect of tacrolimus on anti-proliferation of VSMCs and the expression of calcineurin, NFATc4, and IL-2, we performed cell culture experiments. The cell number of the tacrolimus group was significantly lower than that of the control group at 48 h of incubation (tacrolimus group: 12533 ± 176 cells versus control group: 15833 ± 384 cells, $P < 0.05$, Fig. 5A). Densitometric analysis of Western blotting demonstrated that tacrolimus significantly decreased the expression of calcineurin, NFATc4, and IL-2 of cultured VSMC (Fig. 5B and C). We confirmed that tacrolimus inhibited VSMC proliferation and the calcineurin/NFAT pathway *in vitro*.

We confirmed that calcineurin-siRNA but not control-siRNA decreased the protein expression of calcineurin, NFATc4, and IL-2 of cultured VSMC, and demonstrated that the cell number of the VSMCs transfected with calcineurin-siRNA was significantly lower

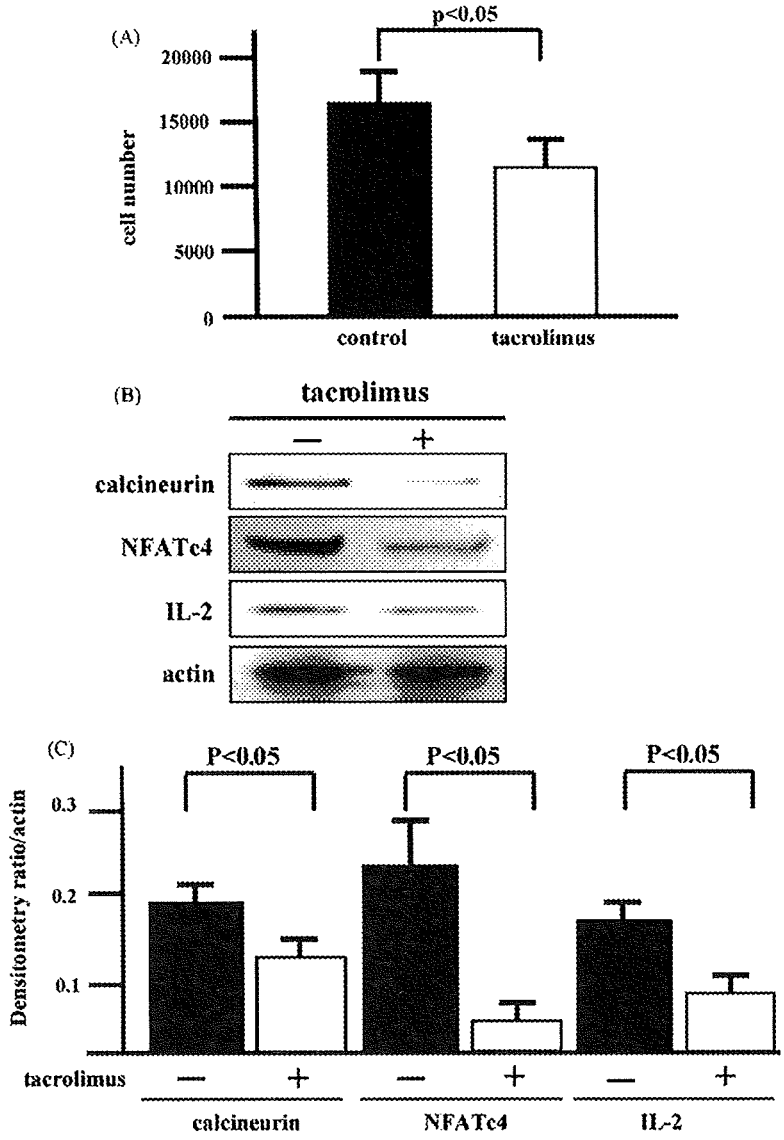

Fig. 5. Cell proliferation at 48 h of incubation with or without tacrolimus and Western blotting for calcineurin, nuclear factor of activated T cell (NFAT), and interleukin-2 (IL-2) of cultured vascular smooth muscle cells (SMCs). (A) The cell number of the tacrolimus group was significantly lower than that of the control group. (B) Representative Western blot analysis for calcineurin, NFATc4, IL-2, and actin in the tacrolimus or control group. (C) Densitometric analysis of Western blotting demonstrated that the expression of calcineurin, NFATc4, and IL-2 in the tacrolimus group was significantly lower compared with the control group.

than that of the VSMCs transfected with control-siRNA (calcineurin-siRNA: 4731 ± 106 cells versus control-siRNA: 7411 ± 233 cells, $P < 0.005$, Additional Fig. 2).

4. Discussion

In this porcine coronary model, the histomorphometric analysis demonstrated that the newly developed TES reduced the neointimal area by 29% compared with a BMS at 12 weeks after stenting. Immunohistochemical staining showed that the expression of calcineurin was up-regulated in neointima and media after stenting, and TES inhibited this up-regulation. In addition, Western blotting demonstrated that the expression of calcineurin, NFATc4, and IL-2 with the TES was lower than with the BMS. Furthermore, in cell culture experiments, we confirmed that tacrolimus and calcineurin-siRNA decreased cell growth and the expression of calcineurin, NFATc4, and IL-2 of cultured VSMCs. It was reported that cyclosporine A suppressed balloon injury-induced neointima formation in a rat carotid artery model by blocking NFAT activation [22]. To the best of our knowledge, this is the first report demonstrating that TES inhibited neointimal formation after stenting via the calcineurin/NFAT signaling pathway.

Intimal thickening and constrictive remodeling are the main elements responsible for restenosis after PCI, and excessive proliferation of VSMCs plays a key role in this process [23]. A previous study reported that tacrolimus inhibited VSMC proliferation [16]. Our cell culture experiments also demonstrated the anti-proliferative effect of tacrolimus on VSMCs. Therefore, we believe that the anti-proliferative effect of tacrolimus on VSMCs results in a reduction of neointimal hyperplasia after stenting.

In activated T cells, tacrolimus and FKBP forms a pentameric complex with calmodulin and calcineurin. Then, this complex inhibits NFAT that is required for activation of the IL-2 gene [14]. Our results of Western blotting demonstrated for the first time that

TES decreased the expression of calcineurin, NFATc4, and IL-2 in the neointima after stenting. Cell culture experiments also revealed that tacrolimus and calcineurin-siRNA decreased the expression of these molecules in cultured VSMCs. Although both tacrolimus and sirolimus bind to FKBP12, the tacrolimus–FKBP complex has been shown to target calcineurin, whereas the sirolimus–FKBP complex targets mammalian target of rapamycin (mTOR) [24]. Therefore, calcineurin may be an important molecular target to prevent restenosis after stenting.

Delayed endothelialization after implantation of a SES or PES may lead to myocardial infarction and death as a result of late stent thrombosis [25,26]. Tacrolimus was reported to have less antiproliferative effect on cultured endothelial cells compared with sirolimus [16]. Therefore, a TES may demonstrate less inhibition of re-endothelialization after implantation than a SES or PES. In our porcine coronary model, the degree of re-endothelialization was similar with TES and BMS. Therefore, tacrolimus is suggested to be a promising compound for the next generation of DESs.

Clinical studies of DESs sometimes show results that are different from the experimental results of long-term porcine studies. The main difference can be explained on the basis of preclinical studies performed in juvenile animals without underlying atherosclerosis [27]. Therefore, a prospective clinical study should be performed in order to evaluate the effect of the newly developed TES on human coronary stenosis.

As the drug is uniformly distributed in the polymer layer in the TES, tacrolimus will be released continuously for several months and completely disappear concomitantly with poly DL-lactate-co-glycolide degradation. The coating method of the TES is completely different from a TES that was developed previously (Janus™, Sorin Biomedica Cardio s.r.l., Saluggia, Italy). The Janus™ stent does not have any polymer vehicle, but has deep reservoirs containing tacrolimus on the external abluminal stent surface; and thus, controlled drug release is difficult. Consequently, the vessel wall may acutely be exposed to an extremely high concentration of the drug, leading to excessive inflammation in the vascular wall. An animal study showed that the tacrolimus concentration in the arterial wall peaked a few days after Janus™ stent implantation and then steeply fell to steady values. These release kinetics may partially explain why the Janus™ stent had a neutral effect on restenosis as shown in the Jupiter II trial [28] and a prospective two-center registry in high-risk patients [29]. Although the same drug was used, the TES may be more useful than the Janus™ stent, because the stent design results in controlled drug release [13].

In conclusion, tacrolimus decreased the proliferation of VSMCs and reduced the expression of calcineurin, NFATc4, and IL-2 in VSMCs. In addition, the newly developed TES inhibited neointimal hyperplasia after stenting via the calcineurin/NFAT/IL-2 signaling pathway, which is one of several mechanisms through which TES inhibits restenosis. Calcineurin may be an important molecular target to prevent restenosis after stent implantation.

Acknowledgments

We wish to thank Mr. Takuji Nishide and Mr. Kohei Fukaya (Kaneka Corporation) for the generous supply of TES and BMS.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.atherosclerosis.2009.07.040.

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特集 妊娠分娩と脳神経外科疾患

もやもや病における硬膜外麻酔下無痛分娩

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髙橋 淳[*2] 飯原弘二[*2] 宮本 享[*2]

Intrapartum Epidural Analgesia for Patients with Moyamoya Disease

by
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There remains some controversy regarding the delivery rout, via cesarean section or vaginal, for pregnant patients with Moyamoya disease. We have set vaginal delivery under epidural analgesia as the first choine of delivery rout in our department of perinatology, at the National Cardiovascular Center. Since 1982, we have experienced 23 deliveries in patients with Moyamoya disease for 26 years. Three cases were transferred after brain hemorrhagic stroke, of whom one pregnant women finally died. Of the remaining 20 cases, 16 (80%) received epidural analgesia and resulted in successful deliveries without complications for mothers and infants. In the other 4 cesarean cases, all of the indication for cesarean were obstetrical, including 2 cases of preeclampsia complaining at headache. Although our clinical experience seems permissive, it should be noticed that our epidural vaginal policy can only be applied to the limited number of institutes where the obstetrical and neruosurgical departments are well connected.

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Key words : Moyamoya disease, pregnancy, epidural analgesia, delivery
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はじめに

もやもや病は男性よりも女性に好発し，比較的若年にみられる疾患であるため，本疾患合併の妊娠・分娩にしばしば遭遇する[1]．しかし，対照試験などが可能なほど症例数はなく，欧米にはきわめてまれであるため，エビデンスに基づいた管理指針がないのが現状である．臨床的な管理の中で最も問題となる一つが，もやもや病合併妊婦の分娩方法の決定，すなわち経腟分娩か帝王切開分娩のいずれを選択することと，いかなる麻酔法を行うかである．本稿は，一施設としてはわが国で最も多くのもやもや病合併妊娠を取り扱っている国立循環器病センターの過去26年間のデータを基に，この分娩法・麻酔法について述べる．

国立循環器病センターにおける成績

Fig. 1 は，国立循環器病センター周産期科が1982年に開設して以来26年間に経験した脳血管障害合併妊娠（ただし，分娩まで至った例のみ）79例を年別の頻度で

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[*2]同脳神経外科

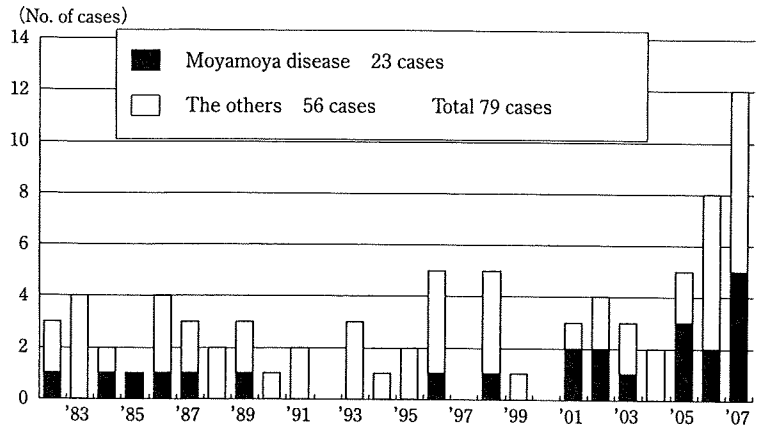

Fig. 1 Cerebrovascular disease-complicated pregnancies 26 years history of the National Cardiovascular Center.

Table 1 Intrapartum epidural analgesia (NCVC)—Important points in informed consent—

- The risk of intracranial bleeding during labor & delivery
- The importance of equilibration of blood pressure in the perinatal period
- Demerit of cesarean section (increase in cesarean rate for next pregnancy, thromboemborism)
- Cesarean delivery vs. Vaginal delivery under epidural analgesia
  Our data in the National Cardiovascular Center
- The possible complications of epidural analgesia
  Frequent use of oxytocin
  Frequent use of vacuum or forceps for delivery
  Complications such as headache, infection, bleeding, toxicity of analgesic agents

Table 2 Intrapartum epidural analgesia (NCVC)—Summary of procedure and technique—

- Nothing per os (NPO)
- Start analgesic during the first stage of labor (cervix dilatation 3-4 cm)
- The initial dose: 1% xylocaine, 3 m*l*+3 m*l*+3 m*l*
- Continuous dose: 0.2% lopivacaine (Anapaine) +2 μg/m*l* fentanest
  About 10 m*l*/hour
- Decreased uterine contraction: oxytocine
- The second stage: outlet forceps or vacuum

示したものである．これまで，年間 1～4 例の症例であったが，最近 2 年間に急増している．これは，脳血管障害合併妊娠がハイリスク妊娠として注目され，高次専門施設へ患者の集約化が行われるようになった傾向を示しているものと考えている．当センターでは，もやもや病合併は 23 例であり，全脳血管障害合併の 29%であった．

国立循環器病センターにおける もやもや病合併分娩の方法

われわれは，禁忌事項などがなければ，原則として硬膜外無痛分娩を選択している．**Table 1** に，インフォームド・コンセントを得る際のポイントを示した．本人，配偶者はもちろんのこと，両親の同席があることが望ましい．特に患者本人の両親は，最悪の事態になった時に，残された子どもの養育を実際に担うことが多く，インフォームド・コンセントの対象者として重要と考えている．

Table 2 に，当センターにおける硬膜外無痛分娩の実際を示した．過期産など産科的な誘発分娩の適応以外は，もやもや病があるのみでは陣痛誘発は行っていない．この場合でも，子宮頚管が開大するなど，分娩準備状態に

Table 3 Cases of epidural vaginal delivery (NCVC)

	Age (y. o.)	Parity	Duration of L & D	Total bleeding (m*l*)
	22	1	7 h 26 min	190
	27	0	48 min	190
	27	1	9 h 3 min	200
	28	1	2 h 37 min	230
	29	1	8 h 19 min	290
	33	2	3 h 43 min	323
	21	0	18 h 57 min	350
	38	2	2 h 46 min	406
	32	1	5 h 43 min	463
	29	0	1 h 17 min	490
	26	0	7 h 52 min	587
	34	0	7 h 1 min	652
	31	3	6 h 42 min	700
	30	2	3 h 55 min	1,030
	30	1	10 h 26 min	1,147
	26	2	2 h 50 min	1,410
Average	28.9	Primi para 5 Multi para 11	5 h 5 min	541 m*l*

Table 4 Cases of cesarean section

Case 1	31 y. o. para 2, Anastomotic bypass surgery 4 times at age 8 y. Her first child, followed in Dep. Neurosurgery of NCVC for Moyamoya dis. Previous 2 deliveries, Cesarean section. Sterilization ope. was added to the elective C/S.
Case 2	32 y. o. para 0, Anastomotic bypass surgery at age 10 y. Hyperthyroidism At 36 wk of gestation, preeclampsia w/headache, emergency C/S
Case 3	37 y. o. para 0, Myoma uteri At 37 wk of gestation, due to failure of progress of labor, emergency C/S
Case 4	35 y. o. para 0, Her mother is also Moyamoya dis. Moyamoya dis. was diagnosed incidentally incidentally during a head examination following a traffic accident At 36 wk of gestation, preeclampsia w/headache, emergency C/S

C/S; cesarean section

あることが必要である．したがって，子宮頸管が未熟な場合は，ラミナリアやバルーンなどで成熟・開大を促進する．麻酔薬には，知覚・運動神経の分別抑制がよく，呼吸抑制が少ないロピバカイン（商品名：アナペイン®）を使用し，子宮収縮の痛みを早期から取るように心掛けている．

国立循環器病センターにおける もやもや病合併分娩の成績

23例の合併妊娠のうち，3例（13%）は妊娠中に脳出血を発症し，他施設から母体搬送となった．年齢はそれぞれ25歳（初産婦），28歳（初産婦），29歳（経産婦）であり，脳出血を発症した妊娠週数は，30週，30週，および36週であった．全例，帝王切開で分娩し，児は生存した．しかし，28歳のケースが母体死亡となった．

それ以外の20例のうち，16例（80%）において硬膜外無痛分娩にて経腟分娩が成功した．**Table 3** に，年齢，経産回数，分娩時間，出血量の内訳を示す．分娩時間や出血量は，正常例の分娩における平均的なものであった．分娩時に，脳の出血性や虚血性の合併症はなく，母児とも予後良好であった．

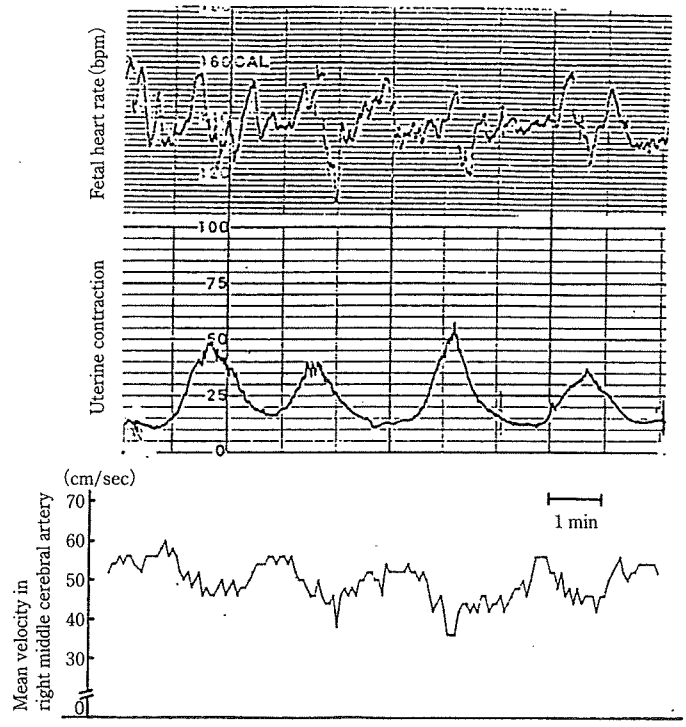

Fig. 2 The change in fetal heart rate, uterine contraction and mean velocity in the right middle cerebral artery in the parturition (First stage of labor, dilatation of cervical canal was 9 cm)[1].

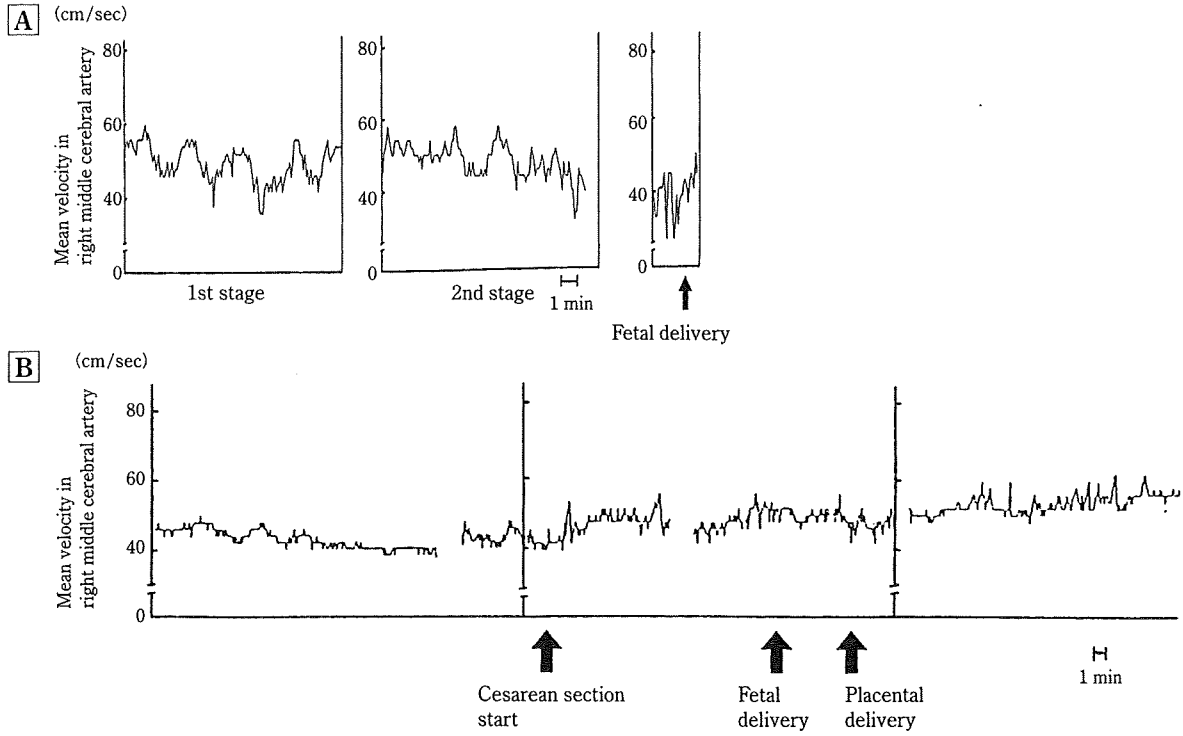

Fig. 3 Mean velocity of Middle cerebral artery in the cases of vaginal and cesarean deliveries[2].

Table 4 に，脳出血以外の適応で帝王切開分娩となった 4 例の概略を示す．2 例は，産科的適応である．他の 2 例（Case 2，4）は，頭痛を伴った重症妊娠高血圧腎症（以前は妊娠中毒症と呼ばれた）例である．妊娠高血圧腎症は，全身の血管内皮の機能異常を中心的な病態とする疾患である．後述するが，脳血管自体が出血や虚血性変化を起こしやすい状態となっていると推定しており，もやもや病症例が妊娠高血圧腎症を伴った場合には，分娩

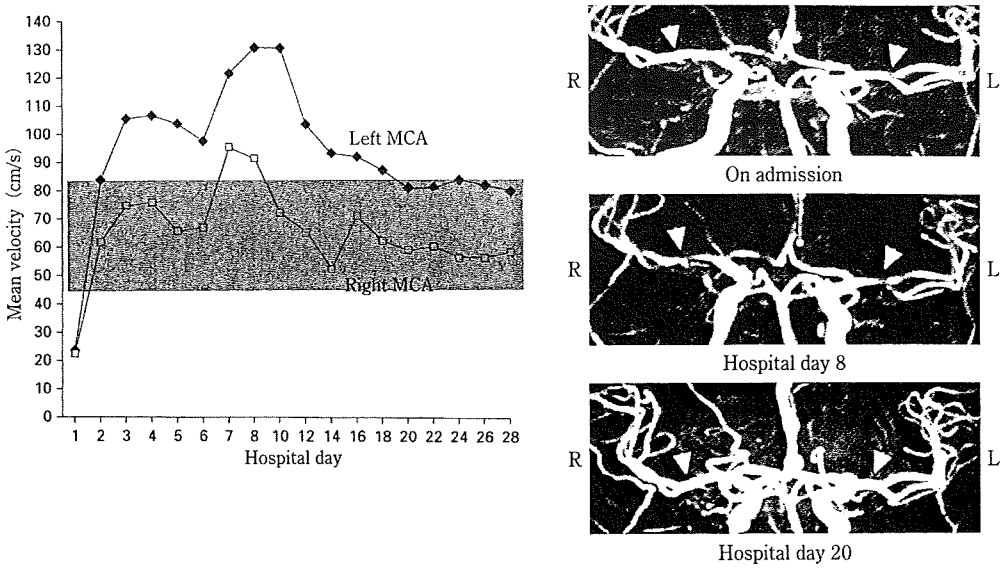

Fig. 4 34 y. o., Postpartum eclampsia[4].

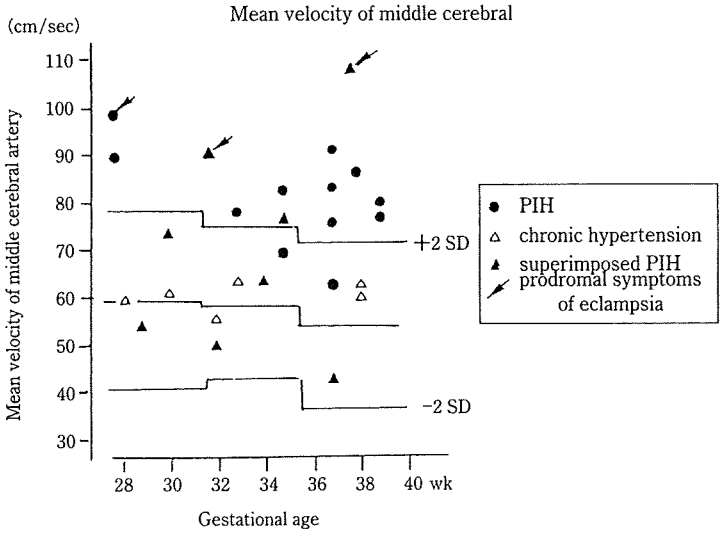

Fig. 5 Individual mean velocity values from 26 hypertensive pregnant women relative to normograms from the middle cerebral artery[3].

方法として帝王切開を選択すべきと考えている．

考　察

1 分娩時の母体脳循環

分娩・陣痛が脳循環に及ぼす影響を考察する．脳循環の指標としては，①脳血流量，②脳血液量，③脳血管に対する shear stress（血流速度）の3つがある．分娩時に脳血流量（または血流速度）が増加すると考えられる因子は，痛みによる血圧上昇と，子宮収縮に伴って子宮から体循環に絞り出されることよる循環血液量と心拍出量の増加である．逆に脳血流量（または血流速度）低下をきたすと考えられる因子は，陣痛時の過換気による低炭酸ガス血症と，怒責（りきみ）による胸腔内圧増加に伴う脳静脈還流低下である．これに，分娩時体位，分娩時出血などが絡んでくると考えられる．

われわれは，非侵襲的でベッドサイドでも測定可能な脳血流測定法である経頭蓋的超音波脳血流速度装置（transcranial Doppler；TCD）を用いて，分娩時に中大脳動脈 M1 部位の血流速度を測定した[1]（Fig. 2）．Fig. 2 の下段に示すように，中段の陣痛曲線のミラーイメージのように変動していることがわかる．陣痛に合わせて約25％の中大脳動脈血流速度の減少が起こった．この時，産婦は怒責をしておらず，この脳血流速度の低下は，陣